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DECLARATION UNDER RULE 132		
	Application #	09/622,199
	Confirmation #	8229
	Filing Date	May 31, 2001
	First Inventor	SCHWARTZ
	Art Unit	1625
	Examiner	Seaman, D. Margaret
	Docket #	P06853US00/BAS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

S I R:

I, Jean-Charles SCHWARTZ, residing at 9 Villa Seurat, 75014 Paris, France, declare and say as:

1. I am a citizen of France.

2. I am honorary Professor and Chairman at Université René Descartes in Paris, honorary member of the Institut Universitaire de France, member of the European Academy (Academia Europea), member of the French Academy of Sciences and author of over 700 publications in international journals.

3. I am an inventor of the above-identified patent application. I am aware that the claims of the present patent application have been rejected for alleged lack of enablement.

4. The present patent application is directed to the treatment of disorders using a compound of formula IIa which acts as a ligand of the histamine H_3 receptor. As a result, the compounds block the H_3 receptor, enhance the release of histamine, increase the level of tele-methylhistamine (a major histamine metabolite), and treat diseases and disorders which benefit from activation of histaminergic neurons activity.

5. I refer to my previous declarations of January 8, 2003 and December 19, 2003 to the effect that experimental results on animals given in the specification and in the latter declaration are predictable of the human activity.

6.1. Clinical conditions in which persons experience loss of Attention, Wakefulness and Memory to a greater extent than one would expect for age may occur between normal aging and Alzheimer disease (AD). However, they do not meet as yet the currently accepted criteria for AD. There are multiple observations indicating that these persons develop AD during the next years in a considerably higher proportion than healthy age-matched persons.

6.2. Although the effects of H_3 receptor antagonists have not yet been studied in persons with either Attention, Wakefulness and Memory disorders or AD, there are good reasons derived from preclinical and clinical experimentations to believe that this novel class of drugs will be useful to treat the symptomatology and the progression of both conditions.

7.1. The size and metabolic activity of histaminergic neurons in the tuberomamillary nucleus and H_1 receptor density in cortex decrease in AD, thus indicating that histaminergic transmission is decreased in AD (Panula et al., *Neurosci.* 1998, 82, 993).

7.2. Additionally, histaminergic neurons have a well-established role in Memorization, Attention and Wakefulness, i. e. cognition (Schwartz and Arrang in *Neuropsychopharmacology*, Davis, Charney, Coyle and Nemeroff eds Lippincott Publ. 2002, pp. 179-190).

Thus, it can be concluded that enhancement of histaminergic neuron activity can compensate cognitive impairment in both AD and Attention, Wakefulness and Memorization Disorders.

7.3. The H_3 receptor is an auto-receptor; blockade of the H_3 receptor enhances the electrical and metabolic activity of histaminergic neurons in the tuberomamillary nucleus and increases histamine release from corresponding terminals (Arrang et al. *Nature*, 1987, 327, 117).

7.4. Enhancement of histamine release from cerebral neurons elicits in animals, e.g. cats or mice, a shift in electroencephalographic (EEG) patterns consisting in the appearance of a higher proportion of high frequency waves at the

expense of low frequency waves; such change in spectral power density is considered as a sign of induction of procognitive effect i.e. enhanced vigilance and attention (Ligneau et al., J.Pharmacol.exper.Ther.,1998,287,658).

7.5. H_3-receptor antagonists are able to exert the above actions (enhancement of activity of histaminergic neurons and increase of histamine release) in order to compensate for the histaminergic transmission impairment in AD and Attention, Wakefulness and Memorization Disorders. To date, they are the sole class of drugs known to exert such actions and therefore constitute a rational treatment of these diseases.

8.1. The impairment of cholinergic neurons in AD is also well established as a cause of the cognitive deficit. The release of histamine from histaminergic neurons triggered by H_3-receptor antagonists onto spared cholinergic neurons is known to enhance the activity of said cholinergic neurons; release of histamine is thus to indirectly improve cognitive functions (Passani et al., *Neurosci. Biobehav. Rev.*, 2000, 24, 107 et Bacciotini et al., *Behav. Brain Res.*, 2001, 124, 183). It is thus expected that H_3-receptor antagonists improve cognitive functions in AD as well as Attention, Wakefulness and Memorization Disorders.

8.2. This is confirmed by experiments showing that H_3-receptor antagonists reverse the learning deficit associated with cholinergic impairment experimentally induced in rodents by drugs like scopolamine (Passani et al., et Bacciotini et al., *ibid.*).

9. This is also confirmed by the References numbered 1, 6, 7, 8 submitted with my previous Declarations. Support for AD can be found in References 1 and 8 (abstract); Support for Attention, Wakefulness and/or Memorization disorders can be found in References 1 (pages 27 and 30), 6 (page 401), 7 (abstract) and 8 (title + abstract).

10.1 Recent personal studies (unpublished) in humans receiving an histamine H_3-receptor antagonist have evidenced a shift towards high frequency waves in the EEG (known to be associated with procognitive effects) as well as on improved attention (assessed by impaired performance in the Critical Flicker Test).

10.2. In these studies, groups of 5 male human volunteers received either a placebo or one compound of the invention (compound of example 117) orally, in capsules, at single dosages of 40, 60, 90 or 120 mg.

Their EEG were recorded at four leads, two anteriorly (L1 and L4) and two posteriorly (L2 and L3) and the recordings submitted to quantitative spectral analysis using Fourier transforms. The data showed a significant shift of the wave frequencies distributions with an increased population of the very high frequency waves (20-30 and 30-40 Hz) at the expense of the low frequency waves. This change appeared to be dose dependent, being manifested by more than 30% increase in the Area Under The Curve describing the frequencies distribution in anterior leads after a dose of 120mg.In addition similar changes were recorded in a group of 6 volunteers receiving the same compound at 40 mg, once a day, for 9 days, the effect being more marked at day 8 than at day 1.

In subjects receiving single doses of the same compound, a dose-dependent improvement of performances was evidenced on the Critical Flicker Fusion Test, a classical psychometric test evaluating the levels of vigilance and attention: for example at 60 mg the Flicker-Fusion threshold was enhanced by ~1.5 Hz after 90 min and by ~2.5 Hz after 11h (P inferior to 0.01).

10.3. These data show that:

1) EEG changes indicate enhanced arousal, vigilance, attention and improved cognition; further, these changes were similar to those reported in animals

2) Psychometric tests confirm these cognitive improvements

3) These cognitive improvements were not only sustained but also augmented after repeated administration, showing the potential of these drugs for treatment of chronic cognitive impairment disorders, such as those occurring in AD and Wakefulness, Attention and Memorization disorders, even in the absence of brain histaminergic neuron activity defects.

11. Consequently, the above discussions and experimental results provided sound evidence that there exists a direct nexus between the H3 receptor inhibiting activity shown by the compounds of the invention and their utility for treating Alzheimer disease and Wakefulness, Attention and Memorization disorders as presently recited in claim 1.

12. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this day of 3 June 2004

J.C. SCHWARTZ

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Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor

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Although histaminergic neurones have not yet been histochemically visualized, there is little doubt that histamine (HA) has a neurotransmitter role in the invertebrate and mammalian central nervous system[1,2]. For example, a combination of biochemical, electrophysiological and lesion studies in rats have shown that histamine is synthesized in and released from a discrete set of neurones ascending through the lateral hypothalamic area and widely projecting in the telencephalon[3-5]. Histamine acts on target cells in mammalian brain via stimulation of two classes of receptor (H_1 and H_2) previously characterized in peripheral organs[6,7] and probably uses Ca^{2+} and cyclic AMP, respectively, as second messengers[8-10]. It is well established that several neurotransmitters affect neuronal activity in the central nervous system through stimulation not only of postsynaptic receptors, but also of receptors located presynaptically which often display distinct pharmacological specificity and by which they may control their own release. Such 'autoreceptors' have been demonstrated (or postulated) in the case of noradrenaline, dopamine, serotonin, acetylcholine and γ-aminobutyric acid (GABA) neurones[11,12] but have never been demonstrated for histamine. We show here that histamine inhibits its own release from depolarized slices of rat cerebral cortex, an action apparently mediated by a class of receptor (H_3) pharmacologically distinct from those previously characterized, that is, the H_1 and H_2 receptors.

We studied histamine release from slices of rat cerebral cortex previously labelled by preincubation in the presence of ^{3}H-histidine. The cerebral cortex was selected for study as histamine synthesis in this region occurs predominantly in terminals of extrinsic neurones as shown by its occurrence in synaptosomes[13], its strong ipsilateral reduction following lesions at the level of the lateral hypothalamic area[3,14] and its almost total interruption after complete cortical deafferentation[15]. Labelling of the endogenous histamine stores by the ^{3}H-labelled precursor[16] instead of the ^{3}H-amine itself (as is performed in most *in vitro* release studies of catecholamines, for instance) was selected because an active re-uptake mechanism analogous to that operating for other amine or amino acid neurotransmitters has never been demonstrated for histamine in brain, which would allow its selective entry into histaminergic neurones. Thus, although brain slices incubated in the presence of ^{3}H-histamine are labelled and thereafter release the ^{3}H-amine when depolarized[17-20], several features make it doubtful that these processes involve a transport system operating selectively within histaminergic neurones: (1) the entry of the labelled amine into cerebral slices is extremely slow, equilibrium not being reached for several hours[17]; (2) the process is not saturable, does not occur against marked concentration gradients and its energy-dependent character has never been demonstrated (refs 17–19 and J.M.A., M.G. and J.C.S., unpublished observations); (3) the extent to which slices from several brain regions are labelled by ^{3}H-histamine varies but does not parallel the distribution of histaminergic neurone markers like endogenous histamine content or L-histidine decarboxylase activity[18].

Cortical slices were preincubated in the presence of 3.3×10^{-7} M ^{3}H-histidine and a series of washing steps were performed to eliminate excess ^{3}H-labelled precursor and to obtain a low constant basal efflux of ^{3}H-histamine. Addition of 30 mM K^+ consistently elicited in 2 min a fivefold increase in the level of ^{3}H-histamine in the medium which, in such conditions, represented ~10% of the total (Table 1). The magnitude of the depolarization-induced release, expressed as a percentage of the tissue content, was similar to that of endogenously synthesized ^{3}H-histamine[16] or of endogenous histamine[21] from hypothalamic slices. However, the K^+-induced release was not accompanied by a stimulation of ^{3}H-histamine formation as that observed[16] in slices to which the depolarizing stimulus was applied while high ^{3}H-histidine levels were still present in the medium; this suggests that, due to the extensive preliminary washings performed in the present experiments, the specific activity of the ^{3}H-precursor in the synthesis pools was reduced to negligible levels compared with those present during the 'synthesis period'. The effect of the 30 mM K^+ stimulus was totally abolished when slices were allowed to synthesize and release ^{3}H-histamine in a Ca^{2+}-free medium (Table 1*a*) or to release it in medium containing 10 mM Mg^{2+}, presumably acting as a Ca^{2+} antagonist (Table 1*b*). The calcium dependency of the depolarization-induced histamine release has also been reported for hypothalamic slices[16,21].

When 10^{-6} M nonradioactive histamine was present in the medium, the ^{3}H-histamine release elicited by 30 mM K^+ (over basal efflux) was inhibited by ~50%, while the basal efflux itself was unaffected (Table 1*c*). The ^{3}H-histamine release induced by another depolarizing agent, veratridine, acting by promoting Na^+ permeability, was also significantly inhibited in

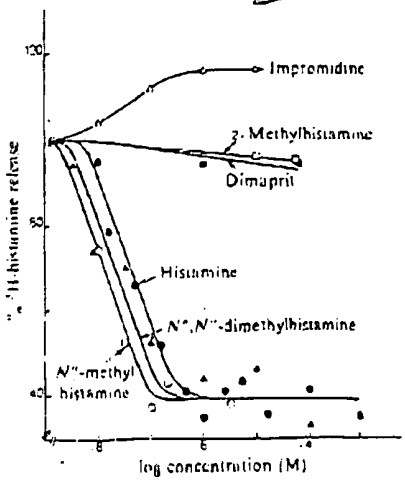

Fig. 1 Effect of various histamine agonists on the K^+-evoked release of ^{3}H-histamine from slices of rat cerebral cortex. Rat cortical slices were prepared, preincubated with ^{3}H-histidine and washed by seven successive transfers as described in Table 1 legend. After the last washing period, 250-μl aliquots of the slice suspension (2.5–3.5 mg protein) were distributed in plastic tubes (Eppendorf) containing 10 μl of the solutions of histamine or agonists (adjusted to *p*H 7.0) and kept at 37 °C. Five minutes later, 250 μl of a modified Krebs–Ringer's solution were added to give a final concentration of either 2 mM or 30 mM K^+ and the incubations were stopped after 2 min by rapid centrifugation. The spontaneous efflux of ^{3}H-histamine into the medium in the presence of 2 mM K^+ represented 3.0 ± 0.2% of the total ^{3}H-amine present in tissue plus medium (that is, 3,648 ± 242 d.p.m. per mg protein in tissue and 111 ± 9 d.p.m. per mg protein in medium). None of the added agents altered this spontaneous efflux (not shown). In the presence of 30 mM K^+ and the absence of added agents, the ^{3}H-histamine level in the medium represented 12.9 ± 0.8% of the total (3,538 ± 292 d.p.m. per mg protein in tissue and 524 ± 50 d.p.m. per mg protein in medium). The K^+-evoked release was calculated in each experiment as the difference between mean ^{3}H-histamine levels in 30 mM K^+ and 2 mM K^+ media respectively (both expressed as percentages of total ^{3}H-histamine) and represented 9.9 ± 0.8% (mean ± s.e.m of 25 experiments). Results are expressed as percentages of this value, each point representing at least triplicate determinations in 1–10 different experiments. The maximal inhibition of 30 mM K^+-evoked ^{3}H-histamine release by exogenous histamine, N^α-methylhistamine and N^α, N^α-dimethylhistamine was 61 ± 3%.

the presence of 10^{-6} M exogenous histamine (Table 1*e*). It is unlikely that these effects of exogenous histamine resulted from an impaired synthesis of ^{3}H-histamine via end-product inhibition[20] because (1) cerebral L-histidine decarboxylase activity in soluble extracts is not modified in the presence of histamine in concentrations as high as 10^{-2} M (refs 16, 22); (2) no significant ^{3}H-histamine formation was occurring in the slices at the time when the depolarizing stimuli were applied as shown by the unmodified tissue levels of ^{3}H-histamine in the presence of α-hydrazinohistidine, an L-histidine decarboxylase inhibitor[16,23]; also the inhibitory effect of exogenous histamine persisted in the presence of the synthesis inhibitor (Table 1*d*). It is also unlikely that the action of exogenous histamine resulted from an isotopic dilution of the endogenously synthesized ^{3}H-histamine in the tissues, because an effect of similar magnitude was still observed when the nonradioactive amine was added together with the depolarizing stimulus (see Fig. 2 legend) or when ^{3}H-histamine was synthesized from ^{3}H-histidine of 250 times lower specific activity (not shown). In contrast, the addition of exogenous histamine does not modify the release process

Table 1 Release of newly synthesized ^{3}H-histamine and its inhibition by exogenous histamine in slices of rat cerebral cortex

Conditions		^{3}H-histamine in tissue (d.p.m. per mg protein)	^{3}H-histamine in medium (d.p.m. per 0.5 ml per mg protein)	^{3}H-histamine in medium (% of total)
a				
Krebs-Ringer's	2 mM K^+	4,940±230	108±7	2.1±0.1
Krebs-Ringer's	30 mM K^+	4,490±170	530±59 (+391%)	10.6±1.1
Krebs-Ringer's without Ca^{2+}	2 mM K^+	10,270±590	200±12	1.9±0.1
Krebs-Ringer's without Ca^{2+}	30 mM K^+	8,780±700	190±15	2.1±0.1*
b				
Krebs-Ringer's	2 mM K^+	3,320±190	106±8	3.1±0.4
Krebs-Ringer's	30 mM K^+	3,190±310	456±56 (+330%)	12.5±1.6
Krebs-Ringer's with 10 mM Mg^{2+}	2 mM K^+	3,427±550	110±8	3.1±0.8
Krebs-Ringer's with 10 mM Mg^{2+}	30 mM K^+	3,405±85	127±8	3.6±0.1†
c				
Krebs-Ringer's	2 mM K^+	3,329±136	123±6	3.5±0.1
Krebs-Ringer's	2 mM K^+ + 10^{-6} M histamine	4,039±367	158±9	3.8±0.5
Krebs-Ringer's	30 mM K^+	2,921±114	588±41 (+378%)	16.4±0.8
Krebs-Ringer's	30 mM K^+ + 10^{-6} M histamine	3,238±120	352±24 (+186%)	9.8±0.5*
d				
Krebs-Ringer's with 0.1 mM α-hydrazinohistidine	2 mM K^+	3,180±110	179±13	5.3±0.2
	30 mM K^+	2,340±90	794±57 (+344%)	25.3±0.7
Krebs-Ringer's with 0.1 mM α-hydrazinohistidine	30 mM K^+ + 10^{-6} M histamine	2,403±299	499±67 (+179%)	17.2±1.1*
e				
Krebs-Ringer's	Veratridine (0 μM)	2,770±130	94±5	3.3±0.2
Krebs-Ringer's	Veratridine (10 μM)	2,560±170	247±10 (+163%)	8.8±0.7
Krebs-Ringer's	Veratridine (10 μM) + 10^{-6} M histamine	2,960±250	193±17 (+105%)	6.1±0.1*

Rats were killed by decapitation and slices (0.3 mm thick) were obtained from the cerebral cortex with a McIlwain tissue chopper. Pooled slices from two animals were washed and resuspended in 12 ml of modified Krebs-Ringer's bicarbonate medium (mM): 120 NaCl, 0.8 KCl, 2.6 $CaCl_2$, 0.67 $MgSO_4$, 1.2 KH_2PO_4, 27.5 $NaHCO_3$, 10 glucose, pH 7.4, gassed with O_2/CO_2 (95:5). The slices (~12 mg protein per ml) were allowed to synthesize ^{3}H-histamine during 30 min incubation at 37 °C (under a constant stream of O_2/CO_2 (95:5)) in the presence of 3.3×10^{-7} M ^{3}H-histidine (41.10^4 d.p.m. ml^{-1}). Before each experiment ^{3}H-histidine (57.5 Ci $mmol^{-1}$; Amersham) was purified by ion-exchange chromatography[13] so that its ^{3}H-histamine content was less then 0.01%. After 30 min, slices were transferred to an open plastic cylinder with a nylon mesh fitted to the bottom as a small basket and washed to remove excess ^{3}H-histidine and to obtain a constant spontaneous ^{3}H-histamine efflux. For this purpose, the basket was successively transferred to seven beakers containing 25 ml of fresh Krebs-Ringer's solution at 37 °C in O_2/CO_2 (95:5) in which it was kept for periods of 4 min (first four washings) and 2 min (last three washings). After the last washing period, 250-μl aliquots of the slice suspension (2.5–3.5 mg protein) were distributed into plastic tubes (Eppendorf) kept at 37°C and containing 250 μl of a modified Krebs-Ringer's solution to give a final concentration of 2 or 30 mM K^+ (*a–d*) (in the presence of 30 mM K^+, NaCl concentration was decreased to compensate, so that isomolarity was maintained), or 10 μM veratridine (*e*). When required, exogenous histamine (10^{-6} M) was present at the start of the incubation. After 2 min, incubations were stopped by rapid centrifugation, pellets were homogenized in 200 μl 0.01 M HCl and ^{3}H-histamine present in the pellet and supernatant was isolated by ion-exchange chromatography on Amberlite CG 50 columns[13]. Radioactivity was estimated by liquid scintillation spectrometry at 45% counting efficiency; at least 1,000 disintegrations were counted over a background of 16 c.p.m. In each experiment, ^{3}H-histamine recoveries and ^{3}H-histidine contaminations in the isolation procedure were evaluated. For this purpose, test ^{3}H-labelled compounds were applied to columns and submitted to the same washing and elution procedures as the extracts of pellet or medium. ^{3}H-histamine recoveries were 86±1% and ^{3}H-histidine contaminations were less than 0.01%; experimental data were corrected accordingly. In a typical experiment with 3 mg of tissue protein, the amount of total radioactivity in slices at the onset of stimulation was 4.5×10^5 d.p.m., indicating that about 95% of the initial ^{3}H-histidine had been removed during the extensive washing procedure. The levels of total radioactivity in tissues or medium were not significantly modified after addition of the various agents. ^{3}H-histamine represented 2.4±0.4% of total radioactivity in the tissue before application of depolarizing stimuli. The amounts of ^{3}H-histamine found in the medium in typical experiments were 370±20 d.p.m. and 1,760±120 d.p.m., in the presence of 2 mM and 30 mM K^+, respectively. In a control experiment the ^{3}H-labelled material released from the slices by a 50 mM K^+ stimulus and eluted from the columns was identified as authentic ^{3}H-histamine by enzymatic transformation into chloroform-extractable ^{3}H-*N*$^\pi$-methylhistamine under the action of semi-purified histamine-*N*-methyltransferase in the presence of 10 μM *S*-adenosylmethionine as a methyl donor[17]; recovery in the organic solvent phase was the same as that for a 1.2×10^{-2} Ci sample of authentic ^{3}H-histamine run in parallel. *a*, A Ca^{2+}-free medium was used from the beginning to the end of the experiment (quadruplicate determinations). The increased ^{3}H-histamine levels in this experiment probably reflect the decrease of release during the successive washing periods[14]. *b*, The final concentration of 10 mM $MgSO_4$ was present only during the 2 min incubation with 2 mM or 30 mM K^+ (triplicate determinations). *c*, Values represent the mean ±s.e.m. of triplicate determinations from 41 experiments except for 2 mM K^+ plus 10^{-6} M histamine (two experiments with quadruplicate determinations). *d*, The last washing period (10 min) and the final 2 min incubation period were conducted in the presence of 10^{-4} M α-hydrazinohistidine (quadruplicate determinations). The total ^{3}H-histamine content (tissue plus medium) was 3,132±120 d.p.m. per mg protein compared with 2,650±131 d.p.m. per mg protein obtained in a parallel experiment in the absence of α-hydrazinohistidine. In contrast, in a control experiment (not shown) the addition of the L-histidine decarboxylase inhibitor 10 min before ^{3}H-histidine resulted in a 93% decrease in ^{3}H-histamine synthesis in the slices (780 versus 11,310 d.p.m.). *e*, One typical experiment with quadruplicate determinations. Similar data were obtained in two additional distinct experiments with 5 μM veratridine (data not shown).

* $P<0.01$; † $P<0.05$ compared with the corresponding controls.

in slices directly labelled with ^{3}H-histamine[4], again indicating that the latter does not significantly enter the endogenous histamine pools. Finally, an inhibition of ^{3}H-histamine inactivation processes by exogenous histamine would have resulted in an opposite effect—that is, an increased recovery in the medium. In fact, the hypothesis that the inhibitory effect of exogenous histamine in slices is a receptor-mediated process is substantiated by observations that it displays characters of saturability, reversibility, high pharmacological specificity and that it is antagonizable in an apparently competitive manner.

The inhibitory action of histamine was concentration-dependent (Fig. 1), with a maximal inhibition of 61 ± 3% of K^+-evoked ^{3}H-histamine release and a half-maximal effect of a $4.1 \pm 0.8 \times 10^{-8}$ M concentration of histamine added 5 min before the K^+ stimulus (when histamine was added together with the K^+ stimulus, this EC_{50} value was increased threefold, while the maximal inhibition was of similar magnitude; see Fig. 2*b*). The effect of exogenous histamine was reversible as inhibition of release was no longer observed when slices were rapidly washed after exposure to the amine and before depolarization (not shown). Because the inhibitory action of exogenous histamine on release was not prevented in the presence of 2×10^{-7} M tetrodotoxin (to block propagation of action potentials in slices), was less pronounced when release was evoked by a stronger depolarization (66 mM K^+ or 20 μM veratridine) and was also observed on cortical synaptosomes depolarized by K^+ (J.M.A., M.G. and J.C.S., in preparation), it is likely to result, as in the case of other neurotransmitters, from a direct stimulation of autoreceptors[11,12].

The inhibitory action of histamine was mimicked by its two N^α and N^τ, N^τ-methyl derivatives (Fig. 1) which showed the same maximal effect but with about three- and twofold higher relative potency, respectively, while they are slightly less potent than histamine at typical H_1 and H_2 receptors (Table 2). In contrast, N^π-methylhistamine or N^τ-methylhistamine (a major histamine metabolite in brain) had no significant inhibitory activity and the same was true for compounds like 2-methylhistamine, 4-methylhistamine, 2-thiazolylethylamine or dimaprit which, however, all display measurable agonist activity at either H_1 or H_2 receptors (Fig. 1 and Table 2). Interestingly, impromidine, a potent but partial histamine agonist at H_2 receptors[23], not only failed to mimick the inhibitory action of histamine (at a concentration between 5×10^{-10} M and 10^{-5} M) but increased by ~20% the K^+-evoked release of ^{3}H-histamine. This facilitatory action of impromidine, although of limited amplitude, was highly reproducible, concentration-dependent (with a half-maximal effect at $\sim 3 \times 10^{-6}$ M) and was not observed on the spontaneous efflux of ^{3}H-histamine (2 mM K^+). This suggested that impromidine might have been acting as an antagonist towards endogenous histamine released by the K^+ stimulus, thus revealing a normal negative feedback modulation of histamine release, as is the case for various autoreceptor antagonists[11,12]. Indeed, the inhibitory action of exogenous histamine (10^{-6} M) on release was progressively reversed in the presence of impromidine at increasing concentrations, with a 50% inhibitory concentration (IC_{50}) of $5.7 \pm 1.1\ 10^{-7}$ M (Fig. 2*a*), about 20 times higher than the drug concentration responsible for the half-maximal facilitatory effect (Fig. 1). The surmountable character of the antagonism of exogenous histamine by impromidine was demonstrated by opposing the drug at fixed concentrations to the amine at increasing concentrations; there was a progressive rightward shift of the concentration-response curve to histamine, whose maximal inhibitory effect was, in all cases, the same—that is, about 60% inhibition of K^+-evoked release (Fig. 2*b*). Schild plot analysis of these data was performed while neglecting the influence of endogenous histamine (inset of Fig. 2*b*) and gave a straight line with a slope of 1.32 ± 0.34, compatible with impromidine acting as a competitive antagonist of exogenous histamine. The pA_2 value of impromidine was 7.5, in good agreement with the K_B value of 6.5×10^{-9} M (Table 2) which is derived from its IC_{50} value in the experiment of Fig. 2*a*, assuming a competitive antagonism[25] and again neglecting the influence of endogenous histamine. Also, a K_B value of 6.3×10^{-9} M for impromidine was found when the drug was opposed to a 10^{-4} M concentration of histamine and its IC_{50} value (4.9×10^{-5} M) was introduced into

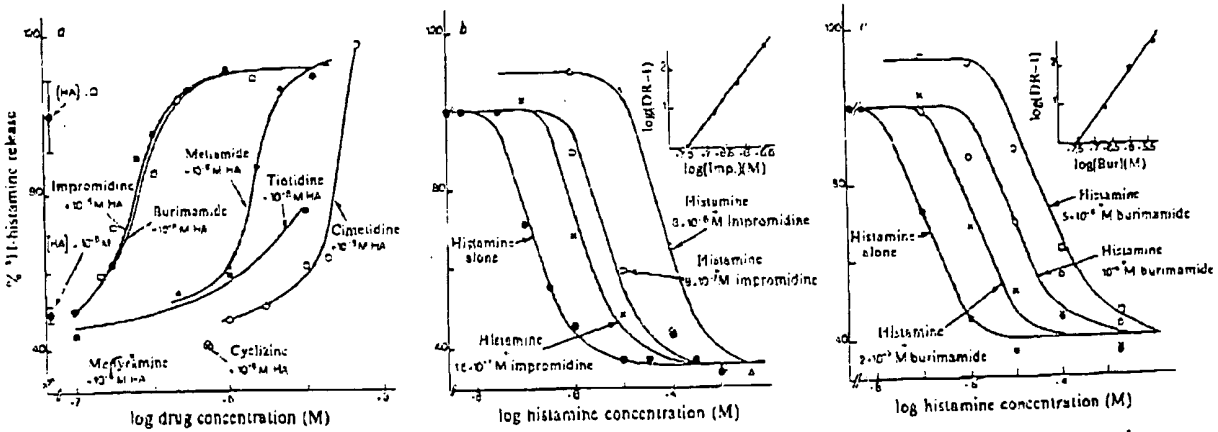

Fig. 2 Reversal by several drugs of the exogenous histamine-induced inhibition of K^+-evoked ^{3}H-histamine release. The procedure was that described in Fig. 1 legend except that exogenous histamine was added, when required, together with the depolarizing Krebs-Ringer's solution, 5 min after the various drugs (added in a 10-μl volume of solution adjusted to *p*H 7). The spontaneous efflux of ^{3}H-histamine (2 mM K^+) was 3.4 ± 0.2% (3,213 ± 154 d.p.m. per mg protein in tissue and 115 ± 8 d.p.m. per mg protein in medium). In the presence of 30 mM K^+ and in the absence of added agents, the ^{3}H-histamine level in medium represented 16.1 ± 1.0% of the total (2,838 ± 125 d.p.m. per mg protein in tissue and 548 ± 48 d.p.m. per mg protein in medium). The results are expressed as percentages of the K^+-evoked ^{3}H-histamine release over spontaneous efflux, in the absence of exogenous histamine ([HA] = 0). *a*, The various drugs in increasing concentrations were opposed to exogenous histamine at a fixed concentration ([HA] = 10^{-6} M) which, alone, inhibited the K^+-evoked release by 50 ± 2% (mean ± s.e.m. of 15 experiments). None of the drugs, at the highest concentration tested, significantly modified spontaneous ^{3}H-histamine efflux. *b*, *c*, Impromidine or burimamide at fixed concentrations were opposed to exogenous histamine at increasing concentrations. The insets represent the Schild plots of data, dose ratios (DR) being determined from the half-maximal inhibitory concentrations of histamine which were estimated graphically considering the total curve for each impromidine (Imp) or burimamide (Bur) concentration. *b*, The slope and 95% confidence limits for the regression of log(DR-1) on log[Imp] was 1.32 (0.98–1.66) and the coefficient of correlation was 0.998. The pA_2 value for impromidine was 7.52. *c*, The slope and 95% confidence limits for the regression of log(DR-1) on log[Bur] was 1.19 (0.47–1.92) and the coefficient of correlation was 0.990. The pA_2 value for burimamide was 7.52. Each point represents the mean of at least triplicate determinations in 1–14 different experiments.

the Cheng–Prussof equation[25] (not shown). Finally, the impromidine concentration ($\sim 3.10^{-8}$ M) required for the half-maximal facilitatory effect (Fig. 1) was close to these apparent dissociation constants. Interpreting this small facilitation as resulting from blockade by impromidine of a feedback inhibition of ^{3}H-histamine release mediated by the same class of receptors as those on which exogenous histamine acts, leads to the idea that the 'equivalent concentration' of endogenous histamine was rather lower than the EC_{50} value of exogenous histamine, that is, 4×10^{-6} M. This would account for an inhibitory effect being still observable when exogenous histamine was added (the 'brake' is not maximally triggered by the endogenous amine).

The consistency of apparent dissociation constants of impromidine in these various tests indicates that neglecting the probably complex influence of endogenous histamine (whose concentration in the vicinity of putative autoreceptors varies with time and is influenced by the antagonists) has probably not biased these evaluations to any great extent. Therefore, although the apparent dissociation constants of antagonists must be regarded, to a certain extent, as approximations (as, inherently, in any other autoreceptor system) they have allowed us to characterize the receptors mediating the feedback inhibition of histamine release.

Table 2. Comparison of potencies of histaminergic agents on the inhibition of cortical ^{3}H-histamine release, the guinea pig ileum contraction (H_1 receptors) and atrial rate (H_2 receptors)

Agent	Inhibition of ^{3}H-histamine release	Ileum contraction	Atrial rate
Relative agonist potency			
Histamine	100	100	100
N^{α}-methylhistamine	<4	0.42	<0.1
N^{τ}-methylhistamine	<4	<0.01	<0.1
N^{α}-Methylhistamine	270	72	74
N^{α}, N^{α}-dimethyl histamine	170	44	51
2-Methylhistamine	<0.08	16.5	4.4
2-Thiazolylethylamine	<0.008	26	2.2
4-Methylhistamine	<0.008	0.23	43
Dimaprit	<0.008	<0.0001	71
Impromidine	<0.03	<0.001	4,810
Antagonist activity (K_B, M)			
Mepyramine	$>5.8 \times 10^{-6}$	4.4×10^{-10}	
Cyclizine	$>5.8 \times 10^{-7}$	1.2×10^{-8}	
D-Chlorpheniramine	$>5.6 \times 10^{-6}$	5×10^{-10}	
L-Chlorpheniramine	$>5.8 \times 10^{-6}$	1.5×10^{-9}	
Metiamide	2.5×10^{-6}	$>10^{-3}$	9.2×10^{-5}
Cimetidine	3.3×10^{-5}	4.5×10^{-3}	7.9×10^{-7}
Burimamide	7×10^{-6}		7.8×10^{-6}
Ranitidine	$>1.2 \times 10^{-4}$		6.3×10^{-6}
Tiotidine	$>1.2 \times 10^{-5}$		1.5×10^{-6}
Impromidine	6.5×10^{-6}		4.6×10^{-7}
SKF 91486	8.8×10^{-6}		2.2×10^{-5}

Histamine and potential agonists were tested (in 2–11 different concentrations) as described in Fig. 1—that is, they were added 5 min before the K^+ stimulus. Their EC_{50} value was defined as the concentration resulting in a half-maximal inhibition of K^+-evoked ^{3}H-histamine release, maximal inhibition being 61 ± 3% (see Fig. 1 legend). The relative potency was calculated as the ratio EC_{50} of histamine/EC_{50} of agonist × 100. The EC_{50} for histamine was $4.1 \pm 0.8 \times 10^{-8}$ M (values obtained by fitting the curve shown in Fig. 1 with an iterative computer least-squares method). Potential antagonists were added in at least four different concentrations 5 min before 10^{-6} M histamine and 30 mM K^+ and their IC_{50}s (concentration for which the inhibitory effect of exogenous histamine was reduced by 50%) determined in experiments similar to those of Fig. 2a using the iterative computer method[34]. Apparent dissociation constants (K_B values) were calculated assuming a competitive antagonism, according to the equation[25]: $K_B = IC_{50}/(1 + s/EC_{50})$, where s represents the concentration of exogenous histamine (10^{-6} M) and EC_{50} the amine concentration ($1.3 \pm 0.2 \times 10^{-7}$ M) eliciting a half-maximal inhibitory effect on K^+-evoked release of ^{3}H-histamine (data from Fig. 2b). None of the agents altered the spontaneous efflux of ^{3}H-histamine at the highest concentration used to determine their antagonist activity. The values defining the activity of the various agents on the guinea pig ileum and atrium are taken from the review of Ganellin[26]. Histaminergic agents were given by C. R. Ganellin. The following agents were inactive as antagonists: scopolamine, spiroperidol, naloxone, propranolol, mianserine, desipramine ($K_B > 10^{-5}$ M), phentolamine, domperidone ($K_B > 10^{-4}$ M) and imidazole ($K_B > 10^{-3}$ M).

While H_1-antihistamines like mepyramine, cyclizine or chlorpheniramine were ineffective in concentrations at which they block H_1 receptors, several H_2-antihistamines antagonized in a concentration-dependent manner the inhibition of release elicited by 10^{-6} M histamine (Fig. 2a and Table 2) and the antagonism was surmounted by 2×10^{-3} M histamine (not shown). Moreover, the competitive nature of the antagonism elicited by the H_2-antihistamine burimamide (Fig. 2c) was checked by Schild plot analysis of the data, giving a straight line with a slope of 1.19 ± 0.73 (inset of Fig. 2c). Again the pA_2 value of 7.5 for this compound was in good agreement with the K_B value of 7×10^{-8} M derived from its IC_{50} value against 10^{-6} M histamine (Table 2). Also, a facilitation of ^{3}H-histamine release similar to that elicited by impromidine was observed in the presence of high concentrations of burimamide (Fig. 2c) as well as of other antagonists (not shown). These effects of H_2-antihistamines clearly cannot be attributed, however, to blockade of H_2 receptors as the potency of the various compounds markedly differed from that displayed at either peripheral or cerebral H_2 receptors[26,27]. Thus, while the apparent dissociation constant of metiamide was in both cases in the micromolar range, large differences were observed for the other compounds: for example, burimamide[7] which is 520 times less potent than tiotidine[28] at H_2 receptors was at least 170 times more potent than the latter at putative histamine autoreceptors. Similarly, SKF 91486 (3[4(5)-imidazolyl]propylguanidine), a weak H_2 receptor antagonist ($K_B = 2.2 \times 10^{-5}$ M)[29] was quite potent at these histamine receptors ($K_B = 8.8 \times 10^{-8}$ M). These differences do not arise from a restricted and variable diffusion of H_2-antihistamines into the slice preparation because: (1) the apparent K_i values of the compounds were not modified when preincubation in their presence was extended from 5 to 15 min; (2) the K_B value of burimamide was lower (by two orders of magnitude) than on H_2 receptors; (3) in guinea pig hippocampal slices of similar thickness, the H_2-antihistamines antagonize the stimulation of cyclic AMP formation mediated by H_2 receptors with potencies in close agreement with those reported on other responses mediated by this homogeneous class of receptors (ref. 30 and J.M.A., M.G. and J.C.S., unpublished data).

Thus, from the relative potencies of histamine agonists and, more reliably[31], from the apparent dissociation constants of histamine antagonists, as well as from the lack of effect of antagonists of other neurotransmitters (Table 2) it can be concluded that the auto-inhibition of histamine release in brain is mediated by an as yet unidentified class of histamine receptor; we propose calling these putative autoreceptors H_3.

A striking feature of the histamine auto-inhibitory process is that it operates at concentrations of the amine at least 100 times lower than those required to stimulate putative postsynaptic receptors in the brain slice preparation, that is, H_1 receptors mediating glycogenolysis[8] or H_2 receptors responsible for the stimulation of cyclic AMP accumulation[9,32]. Although this is not a general feature, there are other examples in the literature of cerebral autoreceptors being more sensitive to the neurotransmitter than corresponding postsynaptic receptors: for example, noradrenaline inhibits its own release via α_2 adrenoreceptor stimulation at concentrations[33] approximately 100 times lower than those required to stimulate β-adrenoreceptors mediating the stimulation of cyclic AMP formation[34]; a similar situation might exist for serotonin[35,36]. It might be difficult at first sight to consider the means of functioning of a synapse in which the release of the neurotransmitter is already inhibited when its concentration in the cleft reaches levels well below the threshold for target cell stimulation. In fact, this is only an apparent paradox that can be resolved by taking into account several features of the system. First, even when maximally stimulated by the exogenous amine, autoreceptors inhibit the release only partially, that is, generally by $\sim 50\%$ as found for histamine, so that transmission can never be totally interrupted by this process. Second, the endogenous amine

might not maximally trigger the auto-inhibitory system, even when its concentration in the cleft is largely above that required to saturate autoreceptors. Thus endogenous histamine maximally inhibited by 20% its own release (see the facilitatory action of impromidine in Fig. 1) although its concentration in the extracellular space of the slices was probably well above the micromolar range (estimation based on instantaneous release of 10% of the endogenous amine into the whole extracellular space of the slices). This incomplete auto-inhibition might result from spatial factors, the released amine not being able to reach before diffusion or inactivation all autoreceptors (like the extrasynaptic ones) that the exogenous amine stimulates. More likely, it might result from temporal factors: the concentration of endogenous histamine corresponding to maximal stimulation of autoreceptors may not be reached within the slices before the short delay beyond which it can no longer affect the release process. The physiological situation, indeed, clearly differs as the release is triggered by trains of impulses so that autoreceptor stimulation during a single impulse may affect the amount of neurotransmitter released by subsequent impulses.

In any event, the high pharmacological specificity of H_3 receptors which clearly differs from that of H_1 and H_2 receptors, suggests that development of compounds able to stimulate or block them selectively will provide useful tools for elucidating the precise functions of histaminergic neurones in the brain.

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Effects of histamine H_3-receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain

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The interaction of the potent histamine H_3-receptor ligands i.e. (R)α-methylhistamine, an agonist, and thioperamide, an antagonist, with the three classes of cerebral histamine receptors was studied in vitro and in vivo. The histamine-induced stimulation of 3',5'-cyclic AMP accumulation in slices of guinea-pig hippocampus was not modified by thioperamide (up to 0.1 mM) and (R)α-methylhistamine stimulated cyclic AMP accumulation only at millimolar concentrations. Hence, both (R)α-methylhistamine and thioperamide were at least 100 000-fold more potent at H_3- than at H_1- or H_2-receptors in brain. In vivo, the turnover of histamine in rat cerebral cortex, as determined from its depletion elicited by α-fluoromethylhistidine in a synaptosomal fraction was not modified by mepyramine and zolantidine but was markedly enhanced by thioperamide at a low dose ($ED_{50} = 2$ mg/kg). Thioperamide also elicited a long-lasting decrease in synaptosomal histamine and increase in radioimmunoassayable N^{τ}-methylhistamine. In contrast, (R)α-methylhistamine markedly reduced cortical [^{3}H]histamine synthesis ($ED_{50} = 5$ mg/kg). This long-lasting action was accompanied by an increase in synaptosomal histamine and a decrease in N^{τ}-methylhistamine levels. These changes were compared with those in plasma drug levels. Hence the two H_3-receptor ligands appear to modify the activity of cerebral histamine neurons markedly and in a long-lasting and opposite manner.

(R)α-Methylhistamine; Thioperamide; Histamine turnover; [^{3}H]Histamine synthesis; N^{τ}-Methylhistamine

1. Introduction

The functional roles of histaminergic neurons in brain have not yet been fully clarified largely because of the lack of pharmacological tools that could be used in behavioral and other in vivo studies to modify their activity selectively (Prell and Green 1986; Schwartz et al., 1986; Hill, 1987; Hough, 1988).

The presence of presynaptic autoreceptors modulating histamine synthesis in, and release from cerebral neurons in vitro by a feedback mechanism was recently evidenced (Arrang et al., 1983; Van der Werf et al., 1987). These receptors, which appear pharmacologically distinct from the H_1- and H_2-receptors mediating the actions of histamine on target cells, were termed H_3-receptors and two potent and selective ligands were designed (Arrang et al., 1987). One is the chiral agonist, (R)α-methylhistamine, nanomolar concentrations of which inhibit [^{3}H]histamine synthesis in, and release from rat brain slices. The other ligand is thioperamide, which antagonizes the actions of (R)α-methylhistamine and histamine at H_3-receptors including those in human brain, with a nanomolar apparent dissociation constant (Arrang et al., 1988). Furthermore, both these compounds were found to be active in vivo in the rat, affecting cerebral histamine turnover in op-

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posite ways, which suggested that they could be used for in vivo studies of the histaminergic system in the CNS (Arrang et al., 1987).

To this aim, we have now assessed the selectivity of these compounds by determining their potencies at cerebral H_1- and H_2-receptors and characterized their in vivo effects in terms of duration and active doses. The former was evaluated for various indices of cerebral histamine turnover. An additional aim of the present study was to compare, in terms of sensitivity and practical feasibility, the various methods that could be used to establish the effects of novel H_3-receptor ligands and other agents affecting histamine neurons in vivo.

2. Materials and methods

2.1. Animals

Male Hartley guinea-pigs (Iffa-Credo, France) weighing 300-350 g were used for measurement of cyclic AMP (cAMP) responses and binding studies. Male Wistar rats (Iffa-Credo, France) weighing 80-110 g were used for all in vivo experiments. Food and water were given ad libitum except in the case of oral treatments, before which rats were fasted for 24 h.

2.2. Determination of cyclic AMP accumulation in brain slices

Slices of guinea-pig hippocampus were prepared and incubated with test agents according to the procedure of Palacios et al. (1978). Incubations were terminated by sonication of the slices and deproteinisation at 95°C for 8 min. The concentration of cAMP was determined by the protein binding assay method of Brown et al. (1971).

2.3. Determination of endogenous histamine

The cerebral cortex was rapidly dissected out in the cold, homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose using a Teflon-glass Potter-Elvehjem homogenizer (clearance 0.10-0.15 mm). A crude P_2 synaptosomal fraction was prepared by means of two centrifugations (1000 × g, 10 min and 20000 × g, 20 min, respectively). The last pellet was resuspended in potassium phosphate buffer (20 mM, pH 7.6) and aliquots were immediately heated at 95°C for 10 min. Centrifugation (5000 × g, 10 min) was followed by assay of the supernatant for histamine. The supernatant could be kept at −80°C for several weeks.

Endogenous histamine was measured with a radioenzymatic assay (Garbarg et al., 1983) using [^{3}H]S-adenosylmethionine (60 nM) and a preparation of rat kidney histamine-N-methyltransferase purified according to Verburg et al. (1983). The purification of the enzyme was slightly modified, with a two-step ammonium sulfate fractionation and the addition of 10% glycerol and 1 mM dithiothreitol in the chromatographic procedures. The enzyme eluted from the phenyl sepharose column was used without further purification. The reaction was stopped after 1 h incubation of the reaction mixture at 25°C by the addition of perchloric acid (final concentration 0.4 N) and N$^\tau$-methylhistamine was extracted in chloroform. The sensitivity of the assay was 5 pg histamine. The recovery of pure histamine added to the tissue extracts was 77 ± 3% and results were corrected accordingly. It was checked that this recovery was not modified in the brain of drug-treated animals.

2.4. Determination of (R)α-methylhistamine in plasma

A radioenzymatic assay was developed, starting from the observation that (R)α-methylhistamine is a substrate for histamine-N-methyl-transferase and its methylated derivative is readily extractible into chloroform (Hough et al., 1981).

Blood was collected from the rats at the time of decapitation. After centrifugation (10000 × g, 2 min) aliquots of the clear plasma were used for assay immediately or kept at −80°C.

(R)α-Methylhistamine was measured in plasma diluted 50- to 100-fold as described above for histamine. Standards of 25-200 pg (R)α-methylhistamine were added to 50- to 100-fold diluted plasma from untreated rats.

When buffer was used this method allowed the detection of 25 pg (R)α-methylhistamine/10 μl.

corresponding to twice the blank values, i.e. the sensitivity of the assay was 5 times less than that for histamine under similar conditions. The blank values with plasma from untreated animals were higher because of the contribution of endogenous histamine (corresponding to a concentration of 93 ± 10 ng/ml) and were subtracted from the values for treated animals. It was assumed, for this assay, that plasma histamine levels were not changed as a result of the drug treatment.

2.5. Determination of endogenous N^{τ}-methylhistamine

The cerebral cortex was homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 N). The clear supernatant was used for assay immediately after centrifugation (5000 × g, 10 min) or was stored at −80 °C.

N^{τ}-Methylhistamine was measured by a new, sensitive and specific radioimmunoassay (Garbarg et al., in preparation). Briefly, N^{τ}-methylhistamine was derivatized with p-benzoquinone (30 mg/ml). The reaction was allowed to proceed at pH 7.6 for 30 min, then 2 M glycine was added to eliminate the excess of benzoquinone. The derivatized extract was mixed with N^{τ}-methylhistamine-benzoquinone-leucine-[^{125}I]tyrosine (10 pM) as a tracer and an antiserum raised in rabbits against N^{τ}-methylhistamine conjugated with bovine serum albumin via p-benzoquinone. After incubation for 16 h at 4 °C, the bound radioactivity was precipitated by the addition of polyethylene glycol and was counted in a gamma spectrometer with an efficiency of 82%. The limit of detection was 5 pg of N^{τ}-methylhistamine. The identity of the immunoreactivity from extracts of cerebral cortex was checked from its co-elution with authentic N^{τ}-methylhistamine on HPLC analysis (C_{18} μBondapak column). The cross-reactivity of histamine in the N^{τ}-methylhistamine assay was only 0.1 %, ruling out any interference of tissue histamine.

2.6. Determination of [^{3}H]histamine synthesis

[^{3}H]L-Histidine (250 μCi) previously purified on Amberlite CG-50 columns was injected in the tail vein of rats 10 min before decapitation. The cerebral cortex was rapidly dissected out and homogenized in 10 volumes (w/v) of ice-cold 0.4 N perchloric acid. The resulting homogenate was centrifuged at 50000 × g, 1 min. A small aliquot of the clear supernatant was used to determine the total radioactivity. Another aliquot was brought up to pH 8.2 and chromatographed on an Amberlite CG-50 column. The ^{3}H amines adsorbed onto the resin were eluted with acetic acid (Garbarg et al., 1983). The chromatographic procedure was repeated a second time to ensure efficient elimination of the ^{3}H precursor, L-histidine. This resulted in a contamination of 0.007% by [^{3}H]L-histidine. The recovery of [^{3}H]histamine was 74 ± 3%. The results were corrected accordingly.

The levels of ^{3}H amines, corresponding mainly to [^{3}H]histamine (Pollard et al., 1974), were expressed either as dpm/g tissue or as the ratio between [^{3}H]histamine synthesized and the total radioactivity in the same extract.

2.7. Determination of L-histidine decarboxylase activity

L-Histidine decarboxylase activity was measured in the crude P_2 synaptosomal fraction using [^{3}H]L-histidine as substrate and a radiochromatographic assay (Garbarg et al., 1983). (R)α-Methylhistamine and thioperamide did not alter histidine decarboxylase activity when they were added to tissue extracts at concentrations up to 3 μM.

2.8. In vitro binding of [^{3}H]mepyramine to H_1-receptors

[^{3}H]Mepyramine binding (Hill et al., 1977) to a particulate fraction from guinea-pig cerebellum was measured as described by Garbarg et al. (1983), using 0.2 μM mianserin to define non-specific binding.

2.9. Radiochemicals and drugs

L-[2,5-^{3}H]Histidine (50 Ci/mmol), S-adenosyl-L-[methyl-^{3}H]methionine (70 Ci/mmol) and [^{3}H]cyclic AMP (8-[^{3}H]adenosine 3'-5'-cyclic phosphate) (30 Ci/mmol) were from Amersham

(U.K.). [^{3}H]Mepyramine (27 Ci/mmol) was from New England Nuclear Corporation (Boston, USA). (R)- and (S)α-methylhistamine were kindly provided by Dr. W. Schunack (Free University, Berlin, FRG), thioperamide by Dr. M. Robba (Université de Caen, France), dimaprit, cimetidine and zolantidine by Dr. C.R. Ganellin (Smith Kline & French Ltd., UK) and α-fluoromethylhistidine by Dr. J. Kollonitsch (Merck Sharp and Dohme Research Laboratories, Rahway, USA). Mepyramine was provided by Specia (Paris, France). All chemicals were from Sigma (USA) except 1,4-p-benzoquinone, purissimum grade, from Fluka AG (Switzerland). Organic solvents were from Prolabo (France) and polyethylene glycol was from Touzart and Matignon (France).

2.10. Statistical analysis

Statistical evaluation of the results was done with Student's t-test.

3. Results

3.1. Actions of thioperamide and (R)α-methylhistamine at cerebral histamine H_1- and H_2-receptors

The effect of thioperamide was tested on the cAMP accumulation elicited by histamine, a response known to involve the stimulation of both H_1- and H_2-receptors in slices from guinea-pig hippocampus.

Thioperamide, 30 μM and 0.1 mM, did not modify significantly the concentration-response curve for histamine (fig. 1). In addition, thioperamide, 0.1 mM, did not modify the concentration-response curve for the H_2-receptor agonist, dimaprit, on the same model, indicating that its K_i value was superior to 0.1 mM at this receptor (not shown). The specific binding of 0.6 nM [^{3}H]mepyramine to H_1-receptors in membranes of guinea-pig cerebellum was inhibited by only 34% in presence of 0.5 mM thioperamide, indicating a K_i value of this compound in the millimolar range (not shown).

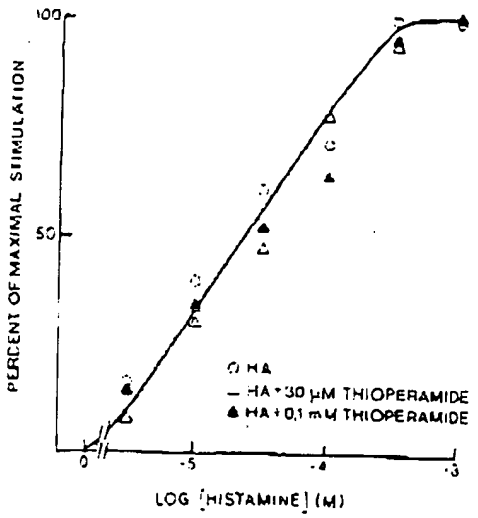

Fig. 1. Effect of thioperamide on the histamine-induced stimulation of cyclic AMP accumulation in slices from guinea-pig hippocampus. Slices were incubated in the presence of histamine for 15 min. When required, thioperamide was added 15 min before. Each point represents the mean of six determinations. The results are expressed as percentage of the maximal response to histamine. The mean basal level of cyclic AMP was 5.8 ± 0.5 pmol/mg protein and was not modified by the addition of thioperamide. The maximal value for histamine stimulation was 473 ± 30%.

The two enantiomers of α-methylhistamine were found to stimulate the accumulation of cAMP in the hippocampal slices and the involvement of H_1- and H_2-receptors in this response was characterized. Hence, the cAMP accumulation elicited by a maximal concentration of either (R)- or (S)α-derivative was completely abolished by the addition of the H_2-receptor antagonist, cimetidine (0.45 mM). In addition, (R)- and (S)α-methylhistamine increased the response to a maximal concentration (0.3 mM) of dimaprit and this increase was completely prevented in the presence of the H_1-receptor antagonist, mepyramine (0.1 μM) (table 1). The effects of the two enantiomers of α-methylhistamine were concentration-dependent in the absence as well as in the presence of dimaprit. The maximal accumulation of cAMP was observed at a concentration of 3 mM for (R)α-methylhistamine and 5 mM for (S)α-methylhistamine when these were added alone to hippo-

TABLE 1

Involvement of H_1- and H_2-receptors in the stimulation of cyclic AMP accumulation elicited by (R)- and (S)α-methylhistamine in slices of guinea-pig hippocampus. Each value is the mean ± S.E.M. of three to six determinations in a typical experiment.

Agent	Cyclic AMP accumulation (pmol/mg protein)
None	2.5 ± 0.3
Histamine	34.1 ± 2.9
(R)α-Methylhistamine (5 mM)	16.7 ± 0.6
(S)α-Methylhistamine (5 mM)	14.4 ± 0.6
(R)α-Methylhistamine + cimetidine (0.45 mM)	2.2 ± 0.5 [a]
(S)α-Methylhistamine + cimetidine (0.45 mM)	2.4 ± 0.5 [a]
Dimaprit (0.3 mM)	11.2 ± 0.5
Dimaprit + (R)α-methylhistamine	20.6 ± 2.1 [b]
Dimaprit + (S)α-methylhistamine	19.0 ± 1.6 [b]
Dimaprit + (R)α-methylhistamine + mepyramine (0.1 μM)	11.3 ± 0.6 [a]
Dimaprit + (S)α-methylhistamine + mepyramine (0.1 μM)	11.3 ± 1.7 [a]

[a] $P < 0.05$ as compared with corresponding values obtained in the absence of antagonist; [b] $P < 0.05$ as compared with dimaprit alone.

campal slices. The maximal accumulation was much lower for both compounds than the maximal stimulation elicited by histamine (4.5 mM) in the same experiment (34.1 ± 5.1 pmol/mg protein).

(R)- and (S)α-methylhistamine when added (both 5 mM) to hippocampal slices in the presence of dimaprit, (0.3 mM), elicited a similar maximal accumulation of cAMP of about 20 pmol/mg protein. Again, the accumulation was much lower than that with histamine (4.5 mM) measured in the same experiment (table 1). The intrinsic activities of (R)α-methylhistamine and (S)α-methylhistamine were calculated, assuming that half of the maximal response to histamine, measured in the same experiment, was due to stimulation of H_1-receptors (Palacios et al., 1978). The intrinsic activity of (R)α-methylhistamine and (S)α-methylhistamine at H_2-receptors (in the absence of dimaprit) was calculated by direct comparison with the maximal response to histamine, assuming that the two acted similarly (table 2). The EC_{50} values were also determined (table 2) and were used to calculate potencies relative to that of histamine based on the EC_{50} of histamine acting at H_1- and H_2-receptors (Palacios et al., 1978). The two enantiomers of α-methylhistamine had similar potencies at H_1- and H_2-receptors and were about 100 times less potent than histamine at both receptors.

3.2. Effects of antagonists of H_1-, H_2- and H_3-receptors on the dynamics of cerebral histamine in vivo

The depletion of endogenous histamine induced by α-fluoromethylhistidine, an irreversible L-histi-

TABLE 2

Properties of (R)- and (S)α-methylhistamine derived from analysis of concentration-response curves for cyclic AMP accumulation in slices of guinea-pig hippocampus. The results reported in this table were obtained from two to three different experiments. EC_{50} were calculated from concentration-response curves made with (R)- or (S)α-methylhistamine alone (H_2-receptor-mediated effect) or in the presence of 0.3 mM dimaprit (H_1-receptor-mediated effect). Intrinsic activities for the H_1-receptor-mediated effect were calculated as the ratio of the maximal stimulation elicited by (R)- and (S)α-methylhistamine in the presence of 0.3 mM dimaprit (taking the stimulation elicited by dimaprit as the basal level) to the half-maximal stimulation elicited by histamine. Intrinsic activities for the H_2-receptor-mediated effect were calculated from the ratio of the maximal stimulation of (R)- and (S)α-methylhistamine alone to that of histamine, assuming that the relative participation of H_1- and H_2-receptors in the response to α-methylhistamine was the same as in the histamine response. Relative potencies were calculated as percentages of the ratio of EC_{50} of histamine (14 μM for H_1-receptors and 6 μM for H_2-receptors) to EC_{50} of agonist.

	(R)α-Methylhistamine with dimaprit	(S)α-Methylhistamine with dimaprit	(R)α-Methylhistamine without dimaprit	(S)α-Methylhistamine without dimaprit
EC_{50} (mM)	1.0 ± 0.3	0.8 ± 0.7	0.4 ± 0.1	0.5 ± 0.1
Intrinsic activity (%)	43	43	36	33
Relative potency (%)	1.4	1.8	1.5	1.3

dine decarboxylase inhibitor (Garbarg et al., 1980) in a fraction of rat cerebral cortex enriched in nerve endings was used for evaluating drug effects.

The efficiency of α-fluoromethylhistidine was checked in each animal 40 min after the administration of the histamine synthesis inhibitor (50 mg/kg), by measuring L-histidine decarboxylase activity. Animals that retained an enzyme activity superior to 20% of the control value were discarded (not shown). Under such conditions, the histamine level was decreased by 36% in α-fluoromethylhistidine-treated rats (table 3). Simultaneous treatment with mepyramine (3 mg/kg i.p.) and/or zolantidine (30 mg/kg i.p.), a brain-penetrating H_1-receptor antagonist (Calcutt et al., 1988), did not modify significantly the level of endogenous histamine in α-fluoromethylhistidine-treated rats. In contrast, thioperamide (3 mg/kg i.p.) significantly enhanced the α-fluoromethylhistidine-induced depletion of histamine (table 3).

This effect was used to determine the ED_{50} of thioperamide. No effect of the H_3-receptor antagonist was observed at a dose of 0.5 mg/kg whereas at 5 mg/kg it decreased the endogenous

TABLE 3

Comparison of the effects of mepyramine, zolantidine and thioperamide on the α-fluoromethylhistidine-induced depletion of histamine in rat cerebral cortex. The animals received mepyramine (3 mg/kg i.p.) and/or zolantidine (30 mg/kg i.p.) or thioperamide (5 mg/kg i.p.) 20 min before α-fluoromethylhistidine (50 mg/kg i.p.) and were killed 40 min later. Means ± S.E.M. of six to eight values.

Treatment	Histamine (ng/g protein)
Saline	347 ± 17
α-Fluoromethylhistidine alone	222 ± 17 [a] (−36%)
α-Fluoromethylhistidine + mepyramine	207 ± 25 [a,N.S.] (−40%)
α-Fluoromethylhistidine + zolantidine	206 ± 29 [a,N.S.] (−41%)
α-Fluoromethylhistidine + mepyramine + zolantidine	222 ± 20 [a,N.S.] (−36%)
α-Fluoromethylhistidine + thioperamide	133 ± 5 [b] (−62%)

[a] $P < 0.01$ as compared with saline; [b] $P < 0.01$ as compared with α-fluoromethylhistidine alone. [N.S.] non-significantly different from α-fluoromethylhistidine alone.

TABLE 4

Effect of thioperamide in increasing doses on α-fluoromethylhistidine-induced depletion of histamine and N^τ-methylhistamine in cerebral cortex. In a first series of experiments, the rats were killed 1 h after simultaneous administration of α-fluoromethylhistidine (20 mg/kg i.p.) and thioperamide (i.p.). Histamine was measured in the crude synaptosomal P_2 fraction. In a second series of experiments, the rats were killed 90 min after oral administration of thioperamide alone. N^τ-Methylhistamine in perchloric extracts was radioimmunoassayed in the supernatants. Means ± S.E.M. of 4-30 values.

Treatment	Histamine in α-fluoromethylhistidine-treated rats (ng/g protein)	N^τ-Methylhistamine (ng/g protein)
Saline	459 ± 20	495 ± 22
α-Fluoromethylhistidine	316 ± 19 [a]	
Thioperamide (0.5 mg/kg)	288 ± 35	515 ± 49
Thioperamide (2 mg/kg)	255 ± 30	869 ± 59 [a]
Thioperamide (5 mg/kg)	155 ± 16 [b]	957 ± 86 [a]
Thioperamide (10 mg/kg)	219 ± 10 [b]	1193 ± 96 [a]
Thioperamide (20 mg/kg)	216 ± 11 [b]	

[a] $P < 0.01$ as compared with controls. [b] $P < 0.02$ as compared with α-fluoromethylhistidine alone.

histamine level by 50-60% (table 4), a change not significantly different from the maximal decrease (60 ± 3%) found 2-4 h after treatment with α-fluoromethylhistidine alone (not shown). These results indicate that the ED_{50} of thioperamide on this model is approximately 2 mg/kg. Thioperamide (p.o.) was also found to increase in a dose-dependent manner the level of N^τ-methylhistamine, a major histamine metabolite, in the cerebral cortex of rats not treated with α-fluoromethylhistidine (table 4). The pronounced increase in N^τ-methylhistamine level observed after 5 mg/kg thioperamide was significant after 30 min and lasted at least 6 h before it returned to normal levels after 16 h (fig. 2). In the same animals, this effect was accompanied by a moderate decrease in the histamine level of the synaptosomal fraction, with the same time course (fig. 2).

3.3. Effects of the H_3-receptor agonist, (R)α-methylhistamine on the dynamics of cerebral histamine in vivo

Following the administration of (R)α-methylhistamine (either 10 mg/kg i.p. or 30 mg/kg p.o.)

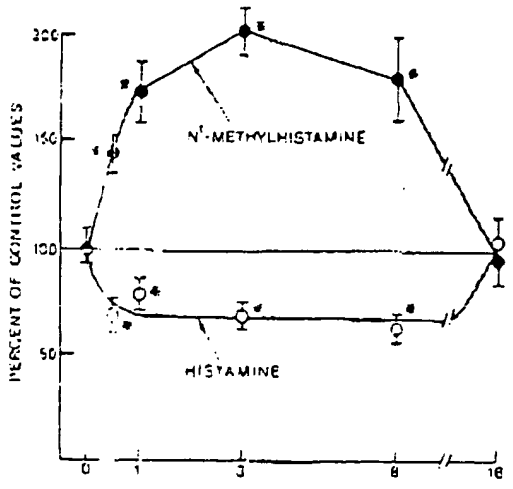

Fig. 2. Effects of thioperamide on histamine and N^{τ}-methylhistamine levels in rat cerebral cortex. The animals were killed at various times after the oral administration of thioperamide (5 mg/kg) or methylcellulose (controls). Histamine was measured in the crude P_2 synaptosomal fraction obtained from a half cortex and N^{τ}-methylhistamine was measured on a perchloric extract from the other half cortex of the same rat. The results are expressed as percent of control values. Control values were 65 ± 23 ng/g protein for histamine and 507 ± 48 ng/g protein for N^{τ}-methylhistamine. Each point represents the mean ± S.E.M. of six values. * $P < 0.01$.

and assuming that the plasma histamine level was unchanged, the drug was detectable for several hours in plasma when a radioenzymatic assay was used. In a typical experiment, 4360 ± 182 dpm/plasma assay was found for untreated rats. After treatment with (R)α-methylhistamine, the dpm/plasma assay were 5551 ± 249 and 5548 ± 330 2 h after i.p. treatment and 4 h after oral treatment respectively, both values being significantly different from that from untreated rats. After parenteral administration of the drug the plasma level decayed in an apparently monophasic manner with an initial half-life of 22 min. After oral administration, the plasma level reached a plateau from 30 min to 2 h, then decreased progressively and was still detectable, although low (0.16 ± 0.09 μg/ml corresponding to a 1.2 μM concentration), after 6 h (fig. 3). The half-life for this decrease was 64 min.

[^{3}H]Histamine synthesis in rat cerebral cortex decreased significantly in a dose-dependent manner 1 h after oral (R)α-methylhistamine whereas total tissue radioactivity was not significantly modified (table 5). There was an about 65% maximal decrease in the synthesis index, i.e. the ratio of [^{3}H]histamine over total radioactivity and this occurred with an ED_{50} of 5.0 ± 1.5 mg/kg. By comparison, the ED_{50} of the drug 1 h after i.p. administration was 1.5 ± 0.6 mg/kg and the maximal reduction in the synthesis index was 75%. The [^{3}H]histamine synthesis index was maximally depressed between 1 and 6 h after oral administration of 10 mg/kg (R)α-methylhistamine and had returned to control levels after 16 h (fig. 4). Similar results were found when the drug was given i.p. (R)α-Methylhistamine at the same dose (10 mg/kg

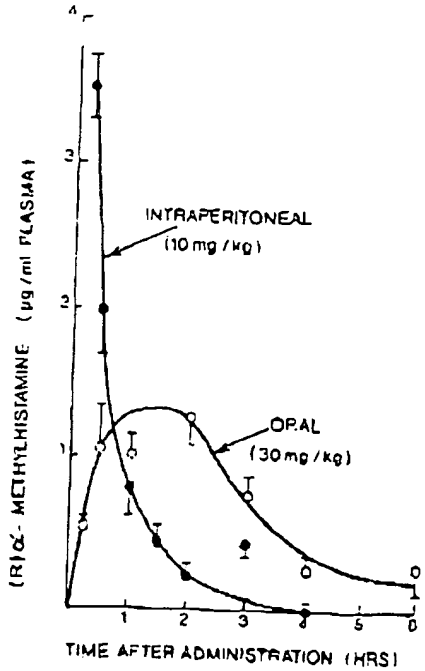

Fig. 3. Drug concentration in plasma of rats treated with (R)α-methylhistamine. The rats were killed at various times after treatment with 10 mg/kg (i.p.) or 30 mg/kg (oral) (R)α-methylhistamine (only the last group was fasted overnight). Plasma levels of the drug were measured in a radioenzymatic assay (see Methods). Each point represents the mean ± S.E.M. of six to eight values.

TABLE 5

Effect of (R)α-methylhistamine in increasing doses on [^{3}H]histamine synthesis in cerebral cortex. The animals were given various doses of (R)α-methylhistamine orally and were decapitated 1 h later. They received [^{3}H]L-histidine (250 μCi in the tail vein) 10 min before being killed. Means ± S.E.M. of 6-16 values.

Treatment	Total radioactivity (dpm/g × 10^{-3})	[^{3}H]Histamine (dpm/g)	[^{3}H]Histamine/total radioactivity (× 10^4)
Saline	35.5 ± 1.7	3639 ± 329	108 ± 7
(R)α-Methylhistamine (1.5 mg/kg)	38.9 ± 2.1	3532 ± 379	87 ± 10
(R)α-Methylhistamine (3 mg/kg)	36.0 ± 2.1	2892 ± 408 *	78 ± 8 *
(R)α-Methylhistamine (10 mg/kg)	31.7 ± 1.0	1143 ± 146 *	38 ± 4 *
(R)α-Methylhistamine (30 mg/kg)	33.7 ± 2.2	1197 ± 168 *	37 ± 6 *

* $P < 0.01$ as compared with saline.

p.o.) decreased N$^\tau$-methylhistamine levels by 38% and this was accompanied by an increased histamine level in the synaptosomal fraction. The percent decrease of the N$^\tau$-methylhistamine level might be underestimated if a metabolite of (R)α-methylhistamine was formed and recognized by the antibodies directed against N$^\tau$-methylhistamine. Both changes were maximal at 3-6 h and were not significant at 1 h (fig. 5). In contrast, the ratio of these two values was significantly reduced at 1 h (22%) and this index was reduced by 47% at 3 h and 51% at 6 h (fig. 5). The changes in this ratio elicited by thioperamide (deduced from data of fig. 2) are reported in the same figure (fig. 5) for comparison.

4. Discussion

The present study confirmed the high pharmacological selectivity of (R)α-methylhistamine and thioperamide toward the H_3-receptor as compared to the two other subclasses of cerebral histamine receptors: at least 10^3 higher concentrations of either the agonist or the antagonist were required for interaction with the H_1- or the H_2-receptors, which both mediate the cAMP response in brain slices (Palacios et al., 1978). The agonist behaved as a partial agonist on this model, as well as on responses mediated by H_1- and H_2-receptors in peripheral tissues (Schunack, 1978; Gerhard and Schunack, 1980), and its potency relative to that of histamine was about 1% whereas it is 1500% at H_3-receptors (Arrang et al., 1987). It may be added that the two compounds did not inhibit cerebral L-histidine decarboxylase activity at 3 μM and, whereas thioperamide was inactive at 0.1 mM on histamine-N-methyltransferase, (R)α-methylhistamine was a substrate (Hough et al., 1981). Although this property has allowed us to develop a radioenzymatic assay for the drug, its K_M is 27 μM (Hough et al., 1981), a concentration

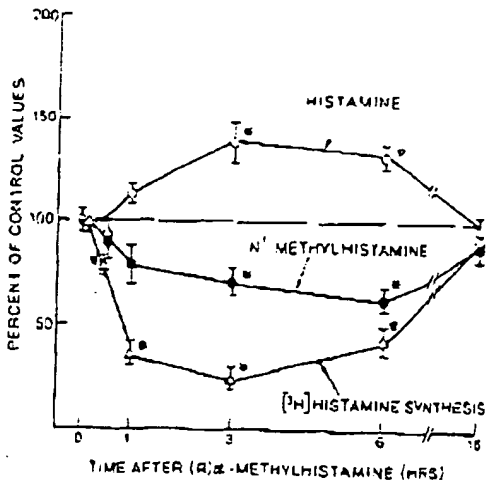

Fig. 4. Effect of (R)α-methylhistamine on histamine and N$^\tau$-methylhistamine levels and [^{3}H]histamine synthesis in rat cerebral cortex. The rats were killed at various times after the oral administration of (R)α-methylhistamine (10 mg/kg) or methylcellulose (controls). Histamine was measured in the crude P_2 synaptosomal fraction obtained from one half cortex and N$^\tau$-methylhistamine was measured on a perchloric extract of the other half cortex of the same rat. Other groups of rats received [^{3}H]L-histidine (250 μCi) in the tail vein 10 min before being killed and the [^{3}H]histamine synthetized was estimated after isolation. The results are expressed as percent of the control values. The control values were: 360 ± 14 ng/g protein for histamine, 529 ± 30 ng/g protein for N$^\tau$-methylhistamine and 3677 ± 217 dpm/g tissue for [^{3}H]histamine. Each point represents the mean ± S.E.M. of 5-17 values. * $P <$

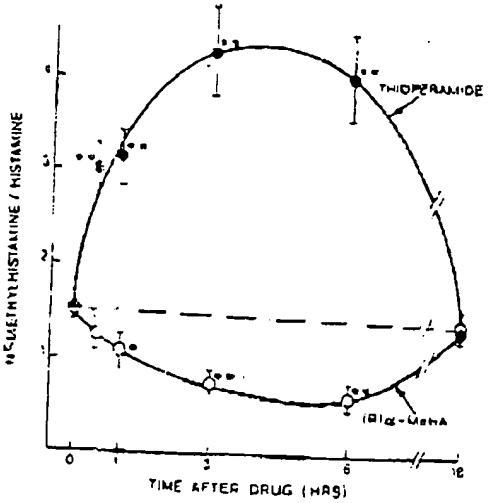

Fig. 3. Effect of (R)α-methylhistamine and thioperamide on the ratio N$^{\tau}$-methylhistamine/histamine as an index of histamine turnover in cerebral cortex. The rats were killed at various times after oral treatment with (R)α-methylhistamine (10 mg/kg), thioperamide (5 mg/kg) or methylcellulose (controls). The ratios were calculated from N$^{\tau}$-methylhistamine and histamine levels taken from the experiments depicted in figs. 2 and 4. * $P < 0.05$, ** $P < 0.001$.

– 10000-fold higher than that required to activate H_3-receptors and, in contrast with histamine (Taylor and Snyder, 1972), (R)α-methylhistamine in a high concentration does not inhibit the enzyme (unpublished observation). The selectivity of (R)α-methylhistamine is also consistent with the fact that its binding (in ^{3}H-labelled form) to cerebral membranes occurs predominantly to pharmacologically characterized H_3-receptors (Arrang et al., 1987).

Blockade of H_1-receptors (in agreement with Pollard et al., 1973a) or H_2-receptors alone or together (table 3) did not significantly modify histamine turnover, as measured by the amine depletion elicited by α-fluoromethylhistidine. Although no direct or indirect agonist of postsynaptic H_1- and H_2-receptors (Garbarg et al., 1978; Schwartz et al., 1986) able to cross the blood-brain barrier is available to fully test this hypothesis, it seems safe to conclude that the activity of histaminergic neurons in the brain is not regulated by long feedback loops involving these receptors.

This contrasts with the clearcut and opposite changes in various indices of histamine turnover elicited by either agonists or antagonists of H_3-receptors.

The very marked thioperamide-induced activation of histamine turnover evidenced on various indices i.e. α-fluoromethylhistidine-induced depletion of endogenous histamine, [^{3}H]histamine synthesis or steady state N$^{\tau}$-methylhistamine level suggests that (i) H_3-receptors regulate selectively the activity of histamine neurons in vivo, (ii) these receptors are tonically activated by endogenous histamine. It is perhaps relevant that the concentration of the amine in the cerebrospinal fluid (and therefore presumably in the extracellular fluid of the brain) is 0.4 μM (Khandelwal et al., 1982) i.e. high enough to stimulate H_3-receptors. Nevertheless, the fact that (R)α-methylhistamine elicited an opposite, although generally less marked, response in the same tests, indicates that H_3-receptors are not maximally activated by the endogenous amine. Interestingly, the steady state level of histamine in the nerve ending-enriched fraction was somewhat modified, with a limited delay and in opposite ways by the two H_3-receptor ligands, indicating that synthesis and release are no longer in a compensational way co-regulated in the absence of normal H_3-receptor control. However, in the case of the agonist, the change in histamine level should be interpreted with some caution in view of the possible cross-reactivity of (R)α-methylhistamine in the radioenzymatic assay. This is, however, unlikely because measurements were performed on a nerve ending-enriched fraction into which the drug is not likely to penetrate.

The present data indicate that the autoreceptor-mediated control of histamine neuron activity constitutes a major regulatory mechanism under physiological conditions. Similar observations were previously reported for α_2-adrenoceptor (Andén et al., 1976; Curet et al., 1987) or 5-HT autoreceptor (Hery et al., 1979; Baumann and Waldmeier, 1984; Pettibone and Pflueger, 1984) ligands in vivo whereas a polysynaptic feedback process seems to control, at least partly, the activity of nigrostriatal dopaminergic neurons (Carlson et al., 1972).

Both thioperamide and (R)α-methylhistamine appear as very useful experimental tools to modify

the activity of cerebral histamine neurons, being active orally at relatively moderate doses (i.e. in the low mg/kg range) and with a markedly long-lasting effect. Thus, with recent, similar observations in mice (Oishi et al., in press), suggests that these drugs might be useful for behavioral and other in vivo studies in several animals species. Nevertheless, the long-lasting central effects of (R)α-methylhistamine contrast somewhat with its rather short initial half-life in plasma i.e. 20 min. Note, however, that the half-life of histamine is much shorter (60 s according to Halpern et al., 1959). In addition, it cannot be excluded that the limited sensitivity of the drug assay with plasma (corresponding to a micromolar detection threshold) does not allow (R)α-methylhistamine to be detected at a time e.g. 6 h, at which its activity at H_3-receptors in brain (which occurs at nanomolar concentrations) is still clearcut.

The various methods we now used to assess drug-induced changes in histamine turnover gave reasonably consistent results. For instance, the ED_{50} values of thioperamide were similar whether obtained from α-fluoromethylhistidine-induced histamine depletion or from the increase in the steady state N^{τ}-methylhistamine level (table 4). In the same way the time course of the effect of (R)α-methylhistamine was the same when changes in endogenous histamine, N^{τ}-methylhistamine levels or [^{3}H]histamine synthesis were measured (fig. 4). However, these methods differ regarding sensitivity and ease of use. The determination of endogenous histamine level in rats pretreated with α-fluoromethylhistidine is the most tedious and the less sensitive method because the possible changes seem rather small and it requires an additional pharmacological treatment and the preparation of a subcellular fraction. Measurement of N^{τ}-methylhistamine by radioimmunoassay is a simple and sensitive method, especially if a drug-induced increase in the catabolite level is expected. Furthermore, the sensitivity of the test can be increased by concomitant determination of histamine and calculation of the ratio of N^{τ}-methylhistamine/histamine. The measurement of [^{3}H]histamine synthesis, a method developed 15 years ago (Poillard et al., 1973b) remains the most sensitive method as shown by the marked effect of (R)α-methylhistamine as compared to that on histamine on N^{τ}-methylhistamine levels (fig. 4). Various other drug treatments were reported to decrease histamine turnover (Oishi et al., 1985; 1986; Baba et al., 1987) but no or non-significant changes in histamine and N^{τ}-methylhistamine steady state levels were observed, i.e. in the absence of α-fluoromethylhistidine or pargyline added to inhibit the formation or the catabolism of these amines.

Hence the present observations show that H_3-receptor ligands elicit very important modifications of histamine turnover as compared to any other drug treatment previously studied. The results also serve to establish the practical conditions for the use of these ligands in behavioral and other studies.

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NEURONAL HISTAMINE DEFICIT IN ALZHEIMER'S DISEASE

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Abstract—Histamine is known to be a neurotransmitter in the brain, but it has not been clearly implicated in major diseases. All histaminergic neurons reside in the posterior hypothalamus and innervate most brain areas, which is compatible with the concept that histamine is involved in general central regulatory mechanisms. A sensitive high-performance liquid chromatographic fluorimetric method was used to measure histamine contents in *post mortem* Alzheimer brains and age-matched controls. The cellular storage sites and distribution of histaminergic nerve fibers were examined with a specific immunohistochemical method. The histamine content was significantly reduced in the hypothalamus (42% of control value), hippocampus (43%) and temporal cortex (53%) of Alzheimer brains. Differences in other cortical areas, putamen and substantia nigra were not significant. Histamine-containing nerve fibers were found in the hippocampus, parahippocampal gyrus and subiculum of both Alzheimer brains and controls. No histamine-containing mast cells were seen in these temporal structures.

Histamine in the human temporal lobe is stored in nerve fibers originating from the posterior hypothalamus, and not in mast cells. Decrease in brain histamine may contribute to the cognitive decline in Alzheimer's disease directly or through the cholinergic system. Development of drugs that penetrate the blood–brain barrier and increase histaminergic activity might be beneficial in Alzheimer's disease. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: hippocampus, tuberomammillary nucleus, hypothalamus, carbodiimide.

Recent studies have shown that histamine may regulate higher brain functions by at least two mechanisms. It enhances hippocampal *N*-methyl-D-aspartate-mediated synaptic currents in cultured hippocampal neurons,[2,26] an effect apparently mediated through the polyamine-binding site on the *N*-methyl-D-aspartate receptor complex,[26] and switches thalamic neuronal activity from rhythmic burst firing to single-spike activity through histamine H_1 and H_2 receptors, thus promoting accurate transmission of thalamocortical relay neurons and processing of sensory inputs and cognition.[12] Thus, histamine mediates its effects not only through specific histamine H_1, H_2 and H_3 receptors (for recent reviews see Refs 9 and 23), but also through modulatory actions on other receptors.

Histamine is widely distributed in the human brain.[10] The cell bodies of histaminergic neurons lie in the posterior hypothalamus, where about 64,000 cells form a loose group in the nucleus tuberomamillaris.[1] These neurons have not been found in other areas of the human brain.[17] The general organization of the histaminergic system is similar in all vertebrates studied so far: cell bodies are found in the tuberomammillary nucleus,[18] which provides almost all parts of the CNS with varicose fibers containing histamine. In the human CNS, histamine-containing projections extend at least to different parts of the cerebral cortex[17] and cerebellar cortex.[19] Other areas of the human brain have not been studied. In Alzheimer's disease, neurofibrillary tangles were found to co-localize with histamine in the tuberomammillary areas in the posterior hypothalamus,[1] and significantly reduced neuron numbers in the tuberomammillary nucleus have been reported in Alzheimer's brains.[15]

Previous studies on histamine in Alzheimer's disease report conflicting results. In one study, significant increases in histamine concentrations were found in almost all brain areas except for the corpus callosum and globus pallidus,[4] whereas another study reported significant decreases in the frontal, temporal and occipital cortices and in the caudate nucleus.[11] The purpose of this study was to analyse the possible changes in the brain histaminergic system using a very sensitive high-pressure liquid chromatographical method and patient material in which the *post mortem* storage times and temperatures are well controlled. As the organization of the human histaminergic system in the temporal lobe has not been analysed, the cellular storage sites of histamine in normal and Alzheimer brains were also revealed

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Abbreviations: PBS, phosphate-buffered saline.

Table 1. Characteristics of patients analysed for histamine content

	Alzheimer's disease	Controls
Age (years)	81.6 ± 9.5 (61–95)	82.2 ± 3.9 (76–88)
Sex (F/M)	7/2	4/1
Post mortem time (h)	17.4 ± 7.2 (6–29)	17.6 ± 7.2 (8–30)

Data are expressed as mean ± S.D.; ranges are given in parentheses.

using immunocytochemistry. This is important, because brain histamine is stored in both neurons and mast cells.[18,20,25]

EXPERIMENTAL PROCEDURES

Samples for histamine measurements were collected from autopsies in the Turku Central University Hospital. A permit for the study was obtained from the National Board of Medicolegal Affairs. The brains were dissected, weighed, frozen on dry ice and kept at −70°C. The patient data are shown in Table 1. For analysis, the samples were analysed using high-performance liquid chromatography fluorometry, as described earlier.[23] On neuropathological examination, the Alzheimer patients showed numerous senile plaques and tangles in the neocortex and hippocampus. All patients fulfilled the requirements for definite Alzheimer's disease according to the CERAD criteria.[16]

For immunohistochemistry, autopsy samples from fresh brains were obtained. Five normal male brains were analysed (age 64.4 ± 14 years, range 49–78, *post mortem* time 44 ± 24 h; cause of death: cardiac infarct). The two Alzheimer brains were also from males (ages 69 and 67 years, *post mortem* times 10.5 and 29 h; cause of death: pneumonia). The brains were dissected into 1-cm-thick slices and fixed by immersion in 4% 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Sigma, St Louis, MO, U.S.A.) and 1% paraformaldehyde in 0.1 M sodium phosphate buffer. After extensive washes in phosphate-buffered saline (PBS), 40-μm-thick free-floating cryostat sections were first treated with 1% hydrogen peroxide in 0.01 M PBS (pH 7.4) for 30 min to remove endogenous peroxidase. To improve the immunostaining the paraformaldehyde was removed by treating the sections with 0.5% sodium borohydride in PBS for 30 min. Sections were then incubated with specific histamine antiserum[17,26] diluted 1:10,000 in PBS containing 0.25% Triton X-100 for 48 h, then washed 2 × 10 min in PBS. Immunoreactivity was visualized with the biotin–streptavidin method (Vectastain Elite Kit, Vector, Burlingame, CA, U.S.A.) according to the manufacturer's instructions. The peroxidase reaction was visualized by incubating the sections in 0.025% diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris–HCl buffer for 30 min. Control sections were treated similarly, except that they were incubated with histamine antiserum preadsorbed with a histamine–ovalbumin conjugate (1–10 μg/ml). No immunoreaction was seen in these samples.

RESULTS

Histamine concentrations

To control the possible effect of *post mortem* time on the histamine content, samples of temporal cortex and hippocampus taken from normal brain on the first *post mortem* day were kept at 4°C, and the histamine content was measured from aliquots taken from these samples each day. A significant, nearly linear but highly variable (70% to 10-fold during six days in different samples) increase occurred after the first day, which called for careful control of *post mortem* times and conditions.

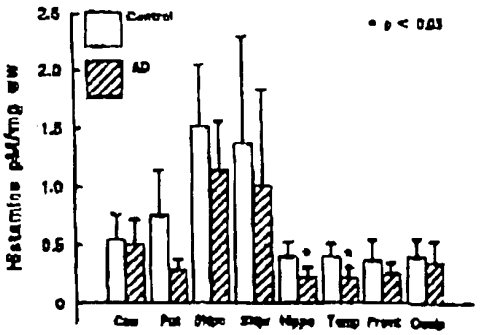

Fig. 1. Histamine concentrations in different brain areas of control brains (n=5) and Alzheimer's disease brains (n=9). Cau, nucleus caudatus; Put, putamen; Hippo, hippocampus; Temp, temporal cortex; SN_{pc}, substantia nigra, pars compacta; SN_{pr}, substantia nigra, pars reticulata; Front, frontal cortex; Occip, occipital cortex.

Table 2. Histamine concentration in the hypothalamus

Alzheimer's disease (n=9)	Controls (n=5)
2.13 ± 1.55	4.97 ± 2.30*

Values given are mean ± S.D. *P<0.05.

Histamine concentrations were significantly lower in the hypothalamus, hippocampus and temporal cortex of Alzheimer brains than in control brains (Fig. 1, Table 2). The differences in the prefrontal and occipital cortices, putamen, pars compacta and pars reticulata of the substantia nigra were not significant, although a tendency to reduced levels was seen. No difference in the concentrations was seen in the nucleus caudatus (Fig. 1).

Distribution of histamine-immunoreactive nerve fibers

All areas of the temporal lobe contained histamine-immunoreactive nerve fibers in normal brains. There was no obvious difference in the morphology or distribution of nerve fibers between normal brains and the two cases of Alzheimer's disease (Fig. 3).

Long varicose fibers immunoreactive for histamine entered the hippocampus via both fimbriae and the perforant pathway (Figs 2, 3). The density of these fibers in the fimbriae and subiculum was higher than in other areas of the hippocampus. Scattered fibers were seen in hippocampal fields CA1–CA3 and in the gyrus dentatus. Moderately dense fibers innervated all layers of the parahippocampal gyrus (Fig. 3c) and entorhinal cortex. No histamine-immunoreactive mast cells or capillary endothelial cells were seen in these brain areas.

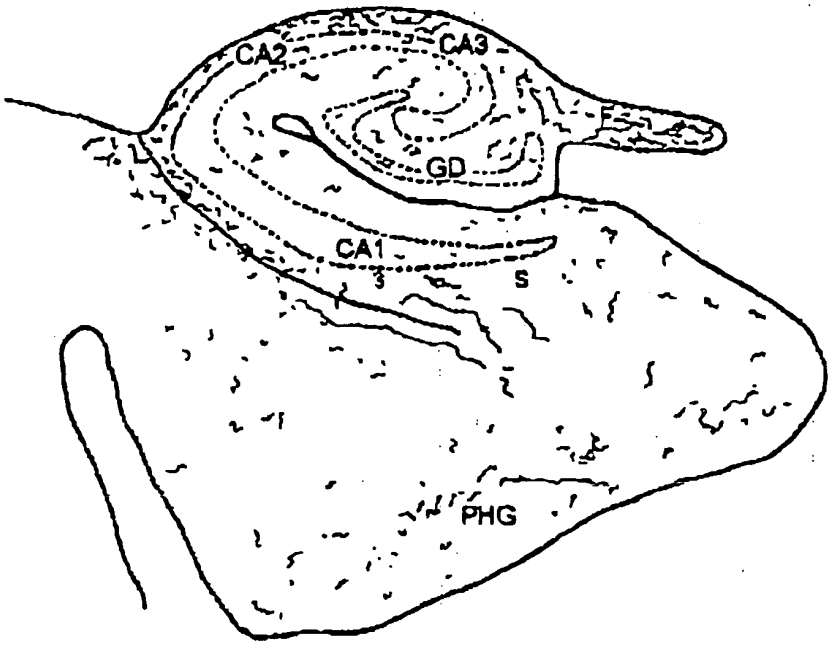

Fig. 2. Schematic distribution of histamine-immunoreactive nerve fibers in normal human hippocampal area. F, fimbriae; CA1–CA3, cornu ammonis, area 1–3; GD, gyrus dentatus; S, subiculum; PHG, parahippocampal gyrus.

DISCUSSION

Based on our findings, nerve fibers are the primary storage site of histamine in the human hippocampus and associated temporal lobe structures. It appears that the hypothalamic histaminergic neurons project to the hippocampus both through the subiculum and fimbriae, which is in agreement with previous findings in the rat and with abundant fiber bundles originating from the hypothalamic histamine-containing neurons in human brain.[1] The results of this study show a clear decrease in histamine concentrations in the hippocampus and temporal cortex in Alzheimer's disease. A similar significant difference was seen in the hypothalamus, where all histaminergic fibers originate.[1,17] Immunohistochemistry was used to reveal the neuronal location of histamine in these structures. Previous studies have shown accumulation of neurofibrillary tangles in the tuberomammillary nucleus and a decrease in neuron numbers in the same area in Alzheimer's disease.[1,15]

The decline in histamine levels observed in this study is in agreement with one previous study,[11] and differs from that of another study.[4] Lack of histamine-immunoreactive mast cells suggests that the observed changes occur in the neuronal pool. Obviously, the *post mortem* time and storage conditions are essential factors that contribute to previous conflicting results, as a significant increase in brain histamine concentration occurs with increasing *post mortem* time. *Post mortem* times need to be carefully controlled.

Although the cholinergic system is commonly considered to be the one most severely affected in Alzheimer's disease, loss of dopamine, serotonin and noradrenaline have also been reported in a number of studies (for review see, e.g., Ref. 6). It is interesting to note that tetrahydroaminoacridine, a non-specific cholinesterase inhibitor that affects cholinergic functions[16] and is found to be beneficial in Alzheimer's disease,[24] also increases the action potential duration of histaminergic neurons,[22] and inhibits histamine *N*-methyltransferase[5] and may thus affect histaminergic transmission in the brain. Decreased histaminergic input may also affect cholinergic activation of cortical and hippocampal neurons, as histamine excites cholinergic nucleus basalis neurons[8] and stimulation of the tuberomammillary histaminergic neurons increases hippocampal acetylcholine release in rats, an effect inhibited by an H_1 receptor antagonist, pyrilamine.[14] The presence of a widespread histaminergic neuronal system in the temporal lobe as shown here and in other parts of the cerebral cortex[17] may also have implications in other disorders involving cortical functions. Improvement of negative symptoms in schizophrenic patients has been reported after adminstration of histamine H_2 receptor blockers,[7] and histamine metabolites in the cerebrospinal fluid of some schizophrenics are elevated.[21]

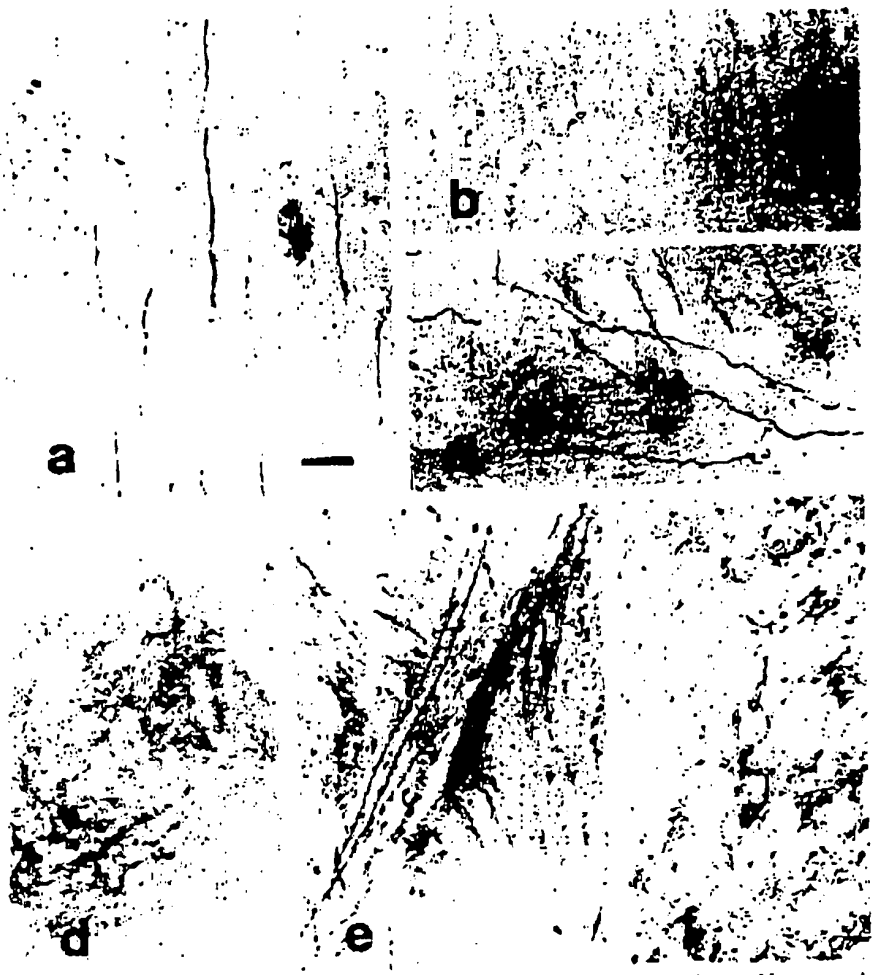

Fig. 3. Immunohistochemical distribution of histamine-immunoreactive fibers in human hippocampal area. Moderately dense fibers in the fimbriae (a), solitary thin varicose fibers in the pyramidal layer of the CA3 area (b) and long, varicose fibers in the subiculum (c) of an Alzheimer brain are shown. A single varicose fiber in the dentate gyrus (d), long varicose fibers in the alveus and subiculum (e), and terminal arborizations of histamine-immunoreactive fibers in the parahippocampal gyrus (f) in normal brain can be seen. Scale bar=50 μm.

CONCLUSION

Taken together, the results show that significant reductions, up to 55% in the hippocampus, are found in brain neuronal histamine content in Alzheimer's disease. It is suggested that lack of brain histamine may contribute to the cognitive decline in Alzheimer's disease. However, activation of different histamine receptors may exert different modulatory effects on other systems. For example, extended use of H_2 blocking agents has been reported to delay the onset of Alzheimer's disease among siblings at high risk.[3]

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14

HISTAMINE

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JEAN-MICHEL ARRANG

In a certain way, histaminergic systems have had a great, although indirect, historical importance in the development of neuropsychopharmacology. Indeed, the discovery of both the neuroleptic agents and the tricyclic antidepressant drugs in the 1950s was derived from the clinical study of behavioral actions of "antihistamines," a class of antiallergic drugs now designated *H_1-receptor antagonists*.

Nevertheless, the histaminergic neuronal system in the brain, although already understood by the mid-1970s, has remained largely unexploited in drug design. Thus, only the traditional brain-penetrating H_1-receptor antagonists, used as over-the-counter sleeping pills, are known to interfere with histaminergic transmissions in the central nervous system (CNS). This situation contrasts with the emergence, in the 1990s, of detailed knowledge of the system that revealed that it shares many biological and functional properties with other aminergic systems overexploited in CNS drug design.

Histamine and its receptors in the brain have been the object of two comprehensive reviews (1,2). Therefore, to limit the length of the present chapter, we have deliberately elected to summarize the detailed information that can be found in these reviews and have added only more recent information and major references.

ORGANIZATION OF THE HISTAMINERGIC NEURONAL SYSTEM

One decade after the first evidence by Garbarg et al. of an ascending histaminergic pathway obtained by lesions of the medial forebrain bundle (3), the exact localization of corresponding perikarya in the posterior hypothalamus was revealed immunohistochemically, and the distribution, morphology, and connections of histamine and histidine decarboxylase-immunoreactive neurons were determined. Data were comprehensively reviewed (4–7), and they are summarized only briefly here.

All known histaminergic perikarya constitute a continuous group of mainly magnocellular neurons (about 2,000 in the rat), located in the posterior hypothalamus and collectively named the *tuberomammillary nucleus* (Fig.14.1). It can be subdivided into medial, ventral, and diffuse subgroups extending longitudinally from the caudal end of the hypothalamus to the midportion of the third ventricle. A similar organization was described in humans, except histaminergic neurons are more numerous (approximately 64,000) and occupy a larger proportion of the hypothalamus (8). Besides their large size (25 to 35 μm), tuberomammillary neurons are characterized by few thick primary dendrites, with overlapping trees, displaying few axodendritic synaptic contacts. Another characteristic feature is the close contact of dendrites with glial elements in a way suggesting that they penetrate the ependyma and come in close contact with the cerebrospinal fluid, perhaps to secrete or receive still unidentified messengers. Neurons expressing mRNAs for histidine decarboxylase (EC 4.1.1.22), the enzyme responsible for the one-step histamine formation in the brain (2), were found by *in situ* hybridization in the tuberomammillary nucleus, but not in any other brain area (9). Tuberomammillary neurons possess the vesicular monoamine transporter 2 (10), which accounts for the histamine-releasing effect of reserpine (2).

The histaminergic neurons are characterized by the presence of an unusually large variety of markers for other neurotransmitter systems: glutamic acid decarboxylase, the γ-aminobutyric acid (GABA)–synthesizing enzyme; adenosine deaminase, a cytoplasmic enzyme possibly involved in adenosine inactivation; galanin, a peptide co-localized with all other monoamines; (Met^5)enkephalyl-Arg^{6}Phe^7, a product of the proenkephalin A gene; and other neuropeptides such as substance P, thyroliberin, or brain natriuretic peptide. Tuberomammillary neurons also contain monoamine oxidase B, an enzyme responsible for deamination of telemethylhistamine, a major histamine metabolite in brain. Finally, a subpopulation of histaminergic neurons is able to take up and decarboxylate exogenous 5-hydroxytryptophan,

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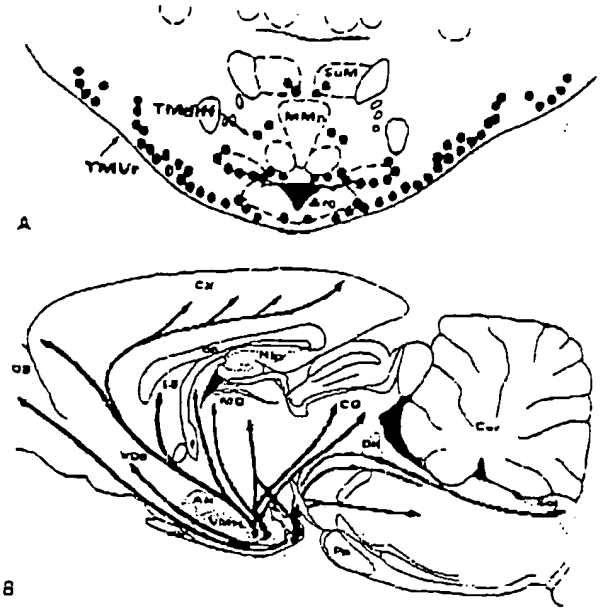

FIGURE 14.1. Localization of histaminergic perikarya *(closed circles)* in tuberomammillary nucleus and disposition of main histaminergic pathways *(arrows)* in rat brain. A: Frontal section of the caudal hypothalamus. B: Sagittal section of the brain. AH, anterior hypothalamic area; Arc, arcuate nucleus; cc, corpus callosum; Cer, cerebellum; CG, central gray; CX, cerebral cortex; DR, dorsal raphe nucleus; f, fornix; Hip, hippocampus; LS, lateral septum; MD, mediodorsal thalamus; MMn, medial mammillary nucleus median part; OB, olfactory bulb; Pn, pontine nuclei; Sol, nucleus of solitary tract; Sox, supraoptic decussation; sum, supramammillary nucleus; TMdiff, tuberomammillary nucleus diffuse part; TMVr, ventral tuberomamillary subgroup rostral part; VDB, nucleus of vertical limb of diagonal band VMH, ventromedial hypothalamic nucleus.

a compound that they do not synthesize, however (5). Discovering the functions of such a high number of putative cotransmitters in the same neurons remains an exciting challenge.

Like other monoaminergic neurons, histaminergic neurons constitute long and highly divergent systems projecting in a diffuse manner to many cerebral areas (Fig.14.1). Immunoreactive, mostly unmyelinated, varicose or nonvaricose fibers are detected in almost all cerebral regions, particularly limbic structures, and it was confirmed that individual neurons project to widely divergent areas. Ultrastructural studies suggest that these fibers make few typical synaptic contacts (6).

Fibers arising from the tuberomammillary nucleus constitute two ascending pathways: one laterally, through the medial forebrain bundle, and the other periventricularly. These two pathways combine in the diagonal band of Broca to project, mainly in an ipsilateral fashion, to many telencephalic areas, for example, in all areas and layers of the cerebral cortex, the most abundant projections being to the external layers. Other major areas of termination of these long ascending connections are the olfactory bulb, the hippocampus, the caudate putamen, the nucleus accumbens, the globus pallidus, and the amygdaloid complex. Many hypothalamic nuclei exhibit a very dense innervation, for example, the suprachiasmatic, supraoptic, arcuate, and ventromedial nuclei.

Finally, a long descending histaminergic subsystem also arises from the tuberomammillary nucleus to project to various mesencephalic and brainstem structures such as the cranial nerve nuclei (e.g., the trigeminal nerve nucleus), the central gray, the colliculi, the substantia nigra, the locus ceruleus, the mesopontine tegmentum, the dorsal raphe nucleus, the cerebellum (sparse innervation), and the spinal cord.

Several anterograde and retrograde tracing studies established the existence of afferent connections to the histaminergic perikarya, namely, from the infralimbic cortex, the septum-diagonal band complex, the preoptic region, the hypothalamus, and the hippocampal area (subiculum) (7, 11). Sleep-active GABAergic neurons in the ventrolateral preoptic nucleus provide a major input to the tuberomammillary nucleus (12,13). Histaminergic neurons also receive very dense orexin innervation originating from the lateral hypothalamus (14). Electrophysiologic studies provided evidence of inhibitory and excitatory synaptic control of tuberomammillary neuron activity by afferents from the diagonal band of Broca, the lateral preoptic area and the anterior lateral hypothalamic area (15). Projections from the brainstem to the tuberomammillary nucleus have also been demonstrated. Retrograde tracing studies combined with immunohistochemistry showed that monoaminergic inputs to the tuberomammillary nucleus originate mainly from the ventrolateral and dorsomedial medulla oblongata and from the raphe nuclei, with a low innervation originating from the locus ceruleus, the ventral tegmental area, and the substantia nigra (16).

MOLECULAR PHARMACOLOGY AND LOCALIZATION OF HISTAMINE RECEPTOR SUBTYPES

Three histamine receptor subtypes (H_1, H_2 and H_3) have been defined by means of functional assays, followed by design of selective agonists and antagonists and, more recently, cloning of their genes (1). All three belong to the superfamily of receptors with seven transmembrane domains (TMs) and coupled to guanylnucleotide-sensitive G proteins (Table 14.1). In addition, histamine affects the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor (17, 18).

Histamine H_1 Receptor

The H_1 receptor was initially defined in functional assays (e.g., smooth muscle contraction) and in the design of po-

TABLE 14.1. PROPERTIES OF THREE HISTAMINE RECEPTOR SUBTYPES

	H_1	H_2	H_3
Coding sequence	491 a.a. (bovine) 488 a.a. (guinea pig) 486 a.a. (rat)	358 a.a. (rat) 359 a.a. (dog, human, guinea pig)	445 a.a. (human) H_{3L} 445 a.a., H_{3S} 415 a.a. (guinea pig) H_{3L} 445 a.a., H_{3S} 413 a.a. (rat)
Chromosome localization	3p25	5	20qTEL
Highest brain densities	Thalamus Cerebellum Hippocampus	Striatum Cerebral cortex Amygdala	Striatum Frontal cortex Substantia nigra
Autoreceptor	No	No	Yes
Affinity for histamine	Micromolar	Micromolar	Nanomolar
Characteristic agonists	2-(3-Trifluoromethyl) histamine	Impromidine Sepromidine	(R)α-Methylhistamine Imetit
Characteristic antagonists	Mepyramine	Cimetidine	Thioperamide Ciproxifan
Radioligands	[^{3}H]Mepyramine [^{125}I]Iodobolpyramine	[^{3}H]Tiotidine [^{125}I]Iodoaminopotentidine	[^{3}H](R)α-Methylhistamine [^{125}I]Iodoproxyfan
Second messengers	Inositol phosphates (+) Arachidonic acid (+) cAMP (potentiation)	cAMP (+) Arachidonic acid (–) Ca^{++} (+)	cAMP (–) Inositol phosphates (–) Arachidonic acid (–) Ca^{++} (–)

gent antagonists, the so-called *antihistamines* (e.g., mepyramine), most of which display prominent sedative properties. Biochemical and localization studies of the H_1 receptor were made feasible with the design of reversible and irreversible radiolabeled probes such as [^{3}H] mepyramine, [^{125}I]iodobolpyramine, and [^{125}I]iodoazidophenpyramine (19,20).

Various intracellular responses were found to be associated with H_1-receptor stimulation: inositol phosphate release, increase in Ca^{2+} fluxes, cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate accumulation in whole cells and arachidonic acid release (1).

The deduced amino acid sequence of a bovine H_1 receptor was disclosed after expression cloning of a corresponding cDNA. The latter was based on the detection of a Ca^{2+}-dependent Cl^- influx into microinjected *Xenopus* oocytes. After the transient expression of the cloned cDNA into COS-7 cells, the identity of the protein as an H_1 receptor was confirmed by binding studies (21).

Starting from the bovine sequence, the H_1 receptor DNA was also cloned in the guinea pig (22), a species in which the pharmacology of the receptor is better established, as well as from several other species including humans (1). Although marked species differences in H_1-receptor pharmacology had been reported (2), the sequence homology between the putative TMs of the proteins is high (90%). The "anatomy" of the H_1 receptor, with a long third intracellular domain and a short C-terminal tail, is similar to that of other receptors positively coupled to phospholipases A_2 and C. Amino acid sequence homology between the TMs of the H_1 and those of the muscarinic receptors (approximately 45%) is higher than between those of H_1 and H_2 receptors (approximately 40%). H_1-receptor antagonists often display significant antimuscarinic activity but only limited H_2-receptor–antagonist properties.

A single gene seems to encode the guinea pig H_1 receptor, and mRNAs of similar size were detected in brain areas and peripheral tissues (22). The structure of the human gene was disclosed (23). Like other receptors of this superfamily, it contains an intron in the 5′ flanking untranslated region, close to the translation initiation codon, but the translated region is intronless.

When stably expressed in transfected fibroblasts, the guinea pig H_1 receptor was found to trigger a large variety of intracellular signals involving or not coupling to pertussis toxin-sensitive G proteins (G_i or G_o), namely, Ca^{2+} transients, inositol phosphates, or arachidonate release (24). H_1 receptor stimulation potentiates cAMP accumulation induced by forskolin in the same transfected fibroblasts, a response that resembles the H_1 potentiation of histamine H_2- or adenosine A_2-receptor–induced accumulation of cAMP in brain slices. All these responses mediated by a single H_1 receptor were known to occur in distinct cell lines or brain slices, but they could have resulted from stimulation of isoreceptors.

Constitutive activity of the recombinant human H_1 re-

ceptor consisting in an agonist-independent increase in inositol phosphates accumulation in COS-7 cells was evidenced. Several H_1-receptor antagonists behaved as inverse agonists (i.e., reduced this constitutive activity), but the physiologic relevance of the process, such as in brain, was not established (25).

The H_1 receptor mediates various excitatory responses in brain (26). A reduction of a background leakage K^+ current was implicated in these responses, in cortical, striatal, and lateral geniculate relay neurons (27,28).

H_1-receptor distribution in the guinea pig brain was established autoradiographically using [^{3}H]mepyramine or the more sensitive probe [^{125}I]iodobolpyramine (20), and the information was complemented by *in situ* hybridization of the mRNA (22). For instance, the high density of H_1 receptors in the molecular layers of cerebellum and hippocampus seems to correspond to dendrites of Purkinje and pyramidal cells, respectively, in which the mRNA is highly expressed. H_1 receptors are also abundant in guinea pig thalamus, hypothalamic nuclei (e.g., ventromedial nuclei), nucleus accumbens, amygdaloid nuclei, and frontal cortex but not in neostriatum (20), whereas they are more abundant in the human neostriatum (29). The H_1 receptor was visualized in the primate and human brain *in vivo* by positron emission tomography using [^{11}C]mepyramine (30).

Blockade of H_1 receptors located in cerebral areas involved in wakefulness and cognition, and including those mediating excitation of thalamic relay neurons (31), neocortical pyramidal neurons (28) and ascending cholinergic neurons (32,33), presumably accounts for the sedative properties of "antihistamines" of the first generation.

Histamine H_2 Receptor

The molecular properties of the H_2 receptor remained largely unknown for a long time. Reversible labeling of the H_2 receptor was achieved using [^{3}H]tiotidine or, more reliably, [^{125}I]iodoaminopotentidine (2).

By screening cDNA or genomic libraries with homologous probes, the intronless gene encoding the H_2 receptor was first identified in dogs (34) and, subsequently, in other species including humans (1). The H_2 receptor is organized like other receptors positively coupled to adenylyl cyclase: it displays a short third intracellular loop and a long C-terminal cytoplasmic tail.

Using transfected cell lines, positive linkage of the H_2 receptor with adenylyl cyclase was confirmed, and unexpected inhibition of arachidonate release and stimulation of Ca^{2+} transients was evidenced (1). Hence H_2 receptor stimulation can trigger intracellular signals either opposite or similar to those evoked by H_1 receptor stimulation. Parallel observations were made for a variety of biological responses mediated by the two receptors in peripheral tissues.

Helmut Haas and colleagues showed that, in hippocampal pyramidal neurons, H_2-receptor stimulation potentiates excitatory signals by decreasing a Ca^{2+}-activated K^+ conductance, presumably by cAMP production (26). H_2-receptor activation depolarizes thalamic relay neurons slightly and increases apparent membrane conductance markedly, responses caused by enhancement of the hyperpolarization-activated cation current I_h (27). In addition to these short-lasting effects, histamine also induces very long-lasting increases in excitability in the CA1 region of the hippocampus through activation of H_2 receptors and the cAMP/cAMP-dependent protein kinase signal transduction cascade. This process is modulated by other receptors such as the H_1 receptor (35).

The sole selective H_2-receptor antagonist known to enter the brain is zolantidine, a compound used sometimes in animal behavioral studies but not introduced in therapeutics (36). However, some tricyclic antidepressants are known to block H_2-receptor–linked adenylyl cyclase potently and interact with [^{125}I]iodoaminopotentidine binding in a complex manner (37).

Autoradiographic localization of the H_2 receptor in guinea pig (20) and human brain (29) shows it distributed heterogeneously. The H_2 receptor is found in most areas of the cerebral cortex, with the highest density in the superficial layers, the piriform, and the occipital cortices, which contain low H_1-receptor density. The caudate putamen, the ventral striatal complex, and the amygdaloid nuclei (bed nucleus of the stria terminalis) are among the richest brain areas. In the hippocampal formation, the relative localizations of the H_2 receptor and its gene transcripts are similar to those observed for the H_1 receptor: the gene transcripts are expressed in all pyramidal cells of the Ammon horn and in granule cells of the dentate gyrus (38), whereas the H_2 receptor is expressed in the molecular layers of these areas, which contain the dendritic trees of the mRNA-containing neurons. The partial overlap with the H_1 receptor may account for their synergistic interaction in cAMP accumulation.

Histamine H_3 Receptor

The H_3 receptor was initially detected as an autoreceptor controlling histamine synthesis and release in brain. Thereafter, it was shown to inhibit presynaptically the release of other monoamines in brain and peripheral tissues as well as of neuropeptides from unmyelinated C fibers (39,40).

Reversible labeling of this receptor was first achieved using the highly selective agonist [^{3}H](R)α-methylhistamine (2), then [^{3}H]N^α-methylhistamine, a less selective agonist, was also proposed (19), as well as, more recently, [^{125}I]iodophenpropit and [^{125}I]iodoproxyfan, two antagonists (41).

The regulation of agonist binding by guanylnucleotides (39), and the sensitivity of several H_3-receptor–mediated

responses to pertussis toxin (42,43), suggested that the H_3 receptor was G_i/G_o protein coupled, a suggestion confirmed by the cloning of the corresponding human (44) and rodent (45) cDNAs. The H_3 receptor gene contains two introns in its coding sequence and several splice variants such as H_{3A} and H_{3S} differing by a stretch of 30 amino acids in the third intracellular loop, were identified (45). The existence of these variants may partly account for the apparent H_3-receptor heterogeneity in binding or functional studies (46).

Significant differences in the pharmacology of the human and rodent H_3 receptor (47) could be assigned to differences in only two amino acid residues in the third TM (48). In various cell lines, stimulation of the H_3 receptor, like that of other G_i-protein–coupled receptors, inhibits adenylate cyclase (44) or phospholipase C (42) and activates phospholipase A_2 (48a).

On neurons, the H_3 receptor mediates presynaptic inhibitions of release of several neurotransmitters, including histamine itself (2,39), norepinephrine, serotonin, dopamine, glutamate, GABA, and tachykinins (40), presumably by inhibiting voltage-dependent calcium channels (39,43).

Several H_3-receptor antagonists, such as thioperamide and ciproxifan, potently enhance histamine release *in vitro* and *in vivo* (2,39,49). This response was originally attributed to blockade of the inhibitory effects of endogenous histamine and was therefore used in many studies, such as behavioral studies, to delineate the functions of histaminergic neurons. However, these drugs were shown to act, in fact, as inverse agonists, and the native H_3 receptor in brain display high constitutive activity including *in vivo* (48a).

Autoradiography of the H_3 receptor in rat (50,51) and monkey brain shows it highly concentrated in the neostriatum, the nucleus accumbens, the cingulate and infralimbic cortices, the bed nucleus of the stria terminalis, and the substantia nigra pars lateralis. In contrast, its density is relatively low in the hypothalamus (including the tuberomammillary nucleus), which contains the highest density of histaminergic axons (and perikarya), a finding indicating that most H_3 receptors are not autoreceptors. In agreement with this concept, intrastriatal kainate strongly decreases H_3 binding sites in the forebrain (as well as in the substantia nigra, consistent with their presence in striatonigral neurons) (50,51). In the human brain, the high densities of H_3 receptors found in the striatum and globus pallidus (29) were lower in patients with Huntington disease, a finding suggesting that the H_3 receptor is also located on striatonigral projection neurons of the direct and indirect pathways (52). Consistent with the proposal that most H_3 receptors are not autoreceptors, a strong expression of H_3-receptor mRNAs was observed not only within the tuberomammillary nucleus, but also in various regions of the rat (44) and guinea pig (45) brain, including the cerebral cortex, the basal ganglia, and the thalamus.

Interaction with NMDA Receptors

Histamine potentiates NMDA-evoked currents in acutely dissociated and cultured hippocampal and cortical neurons, an effect that could not be ascribed to activation of the known histamine receptors (17,18), but rather of a novel recognition site on NMDA receptors containing the subunits NR1A/NR2B (53).

Histamine may play a role in modulating the functions of NMDA receptors *in vivo*. It facilitates the NMDA-induced depolarization of projection neurons in cortical slices (54) and phase shifts the circadian clock by a direct potentiation of NMDA currents in the suprachiasmatic nucleus (55). Histamine, presumably acting through NMDA receptors, facilitates the induction of long-term potentiation and causes long-lasting increases of excitability in the CA1 region of rat hippocampal slices (35).

The histamine-induced modulation of NMDA responses is higher under slightly acidic conditions (56), which occur during hypoxia or epileptiform activity. This may lead to enhancement of neurotransmission or histamine-mediated neuronal death such as that observed in a rat model of Wernicke encephalopathy (57).

HISTAMINERGIC NEURON ACTIVITY AND THEIR CONTROL

Electrophysiologic Properties

Cortically projecting histaminergic neurons share with other aminergic neurons certain electrophysiologic properties evidenced by extracellular recording. They fire spontaneously slowly and regularly, and their action potentials are of long duration (26). Among the pacing events that may contribute to their spontaneous firing, tuberomammillary neurons exhibit a tetrodotoxin-sensitive persistent Na^+ current (58), a Ca^{2+} current probably of the low-threshold type (59), and multiple high-voltage–activated Ca^{2+} currents (43). In addition, they exhibit inward rectification attributed to an I_h current that may increase whole-cell conductance and may decrease the efficacy of synaptic inputs during periods of prolonged hyperpolarization, that is, when histaminergic neurons fall silent (60).

Modulation of Histamine Synthesis and Release *In Vitro*

The autoreceptor-regulated modulation of histamine synthesis in, and release from, brain neurons is well documented (2). It was initially evidenced in brain slices or synaptosomes after labeling the endogenous pool of histamine using the [^{3}H]histamine precursor. Exogenous histamine decreases the release and formation of [^{3}H]histamine induced by depolarization, and analysis of these responses led to the pharmacologic definition of H_3 receptors. The auto-

regulation was found in various brain regions known to contain histamine nerve endings, a finding suggesting that all terminals are endowed with H_3 autoreceptors.

Regulation of histamine synthesis was also observed in the posterior hypothalamus (39), and somatodendritic H_3 autoreceptors inhibit the firing of tuberomammillary neurons (26) by modulating high-voltage–activated calcium channels (43).

Galanin, a putative cotransmitter of a subpopulation of histaminergic neurons, regulates histamine release only in regions known to contain efferents of this subpopulation, that is, in hypothalamus and hippocampus but not in cerebral cortex or striatum (61). In brain slices, galanin also hyperpolarizes and decreases the firing rate of tuberomammillary neurons (26). It is not known, however, whether these galanin receptors behave as "autoreceptors" modulating galanin release from histaminergic nerve terminals, inasmuch as the tuberomammillary nucleus receives a strong galaninergic innervation from the ventrolateral preoptic area (12,13). Other putative cotransmitters of histaminergic neurons failed to affect [^{3}H]histamine release from slices of rat cerebral cortex (62). However, GABAergic inhibitory postsynaptic potentials are mediated by $GABA_A$ receptors located on histaminergic neurons (63). To what extent these receptors play an autoinhibitory role is unclear. A subpopulation of histaminergic neurons contains GABA (5), but the tuberomammillary nucleus also receives dense GABAergic innervation (12,13,15).

[^{3}H]Histamine synthesis and release are inhibited in various brain regions by stimulation of not only autoreceptors but also α_2-adrenergic receptors, M_1-muscarinic receptors, and κ-opioid receptors (2). Because these regulations are also observed with synaptosomes (62), all these receptors presumably represent true presynaptic heteroreceptors. In contrast, histamine release is enhanced by stimulation of nicotinic receptors in rat hypothalamus (64) and by μ-opioid receptors in mouse cerebral cortex (2).

Some molecular mechanisms regulating neuronal histamine dynamics remain unclear. *N*-methylation catalyzed by histamine *N*-methyltransferase is the major process responsible for termination of histamine actions in the brain (2), and genetic polymorphisms for the enzyme have been associated with altered levels of its activity (65). No histamine transporter could be evidenced, and direct feedback inhibition of histidine decarboxylase by histamine has been excluded (2).

Changes in Histaminergic Neuron Activity *In Vivo*

Both neurochemical and electrophysiologic studies indicate that the activity of histaminergic neurons is high during arousal. In rat hypothalamus, histamine levels are low, whereas synthesis is high during the dark period, a finding suggesting that neuronal activity is enhanced during the active phase (2). In mouse cerebral cortex, striatum, and hypothalamus, telemethylhistamine levels are doubled at the end of the dark phase of the cycle as compared with the beginning of the light phase (66). Histamine release from the anterior hypothalamus of freely moving rats, evaluated by *in vivo* microdialysis, gradually increases in the second half of the light period and is maintained at a maximal level during the active phase (67). Such state-related changes are also found in single-unit extracellular recordings performed in the ventrolateral posterior hypothalamus of freely moving cats. Neurons with properties consistent with those of histaminergic neurons exhibited a circadian rhythm of their firing rate, falling silent during deep slow-wave or paradoxical sleep (2). An important determinant of this circadian rhythm of tuberomammillary histaminergic neuron activity is a GABAergic inhibitory pathway originating in the ventrolateral preoptic area and activated during sleep (12,15).

A feeding-induced increase in the activity of histaminergic neurons has also been shown by microdialysis performed in the hypothalamus of conscious rats (68). Histaminergic neurons are a target for leptin in its control of feeding. An enhancement of histamine turnover was observed after intracerebroventricular infusion of leptin (70). Changes in the metabolism and release of histamine observed *in vivo* after occlusion of the middle cerebral artery in rats suggest that the histaminergic activity is also enhanced by cerebral ischemia (71).

Whereas H_1 and H_2 receptors are apparently not involved, inhibition mediated by the H_3-autoreceptor constitutes a major regulatory mechanism for histaminergic neuron activity under physiologic conditions. Administration of selective H_3 receptor agonists reduces histamine turnover (2) and release, as shown by microdialysis (72). In contrast, H_3-receptor antagonists enhance histamine turnover (2,49) and release *in vivo* (73,74), a finding suggesting that autoreceptors are tonically activated.

Agents inhibiting histamine release *in vitro* through stimulation of presynaptic α_2-adrenergic or muscarinic heteroreceptors reduce histamine release and turnover *in vivo*, but systemic administration of antagonists of these receptors does not enhance histamine turnover, a finding suggesting that these heteroreceptors are not tonically activated under basal conditions.

Activation of central nicotinic receptors inhibits histamine turnover (75). Several types of serotonergic receptors are likely to modulate histamine neuron activity. 5-Hydroxytryptamine (5-HT)$_{1A}$–receptor agonists inhibit (76), whereas 5-HT_2–receptor antagonists enhance (77), histamine turnover in various brain regions. Stimulation of D2 (but not D3) dopamine receptors by endogenous dopamine released by amphetamine increases histamine neuron activity (77,78).

Histamine turnover in the brain is rapidly reduced after administration of various sedative drugs such as ethanol, Δ^9-tetrahydrocannabinol, barbiturates, and benzodiaze-

presumably as a result of their interaction with GABA receptors present on nerve endings and on perikarya of histaminergic neurons (63,79).

In contrast, stimulation of μ-opioid receptors enhances histamine turnover in brain (2). NMDA receptors increase *in vivo* release of histamine from the anterior hypothalamus (80). Activation of NMDA and non-NMDA receptors in the diagonal band of Broca, the lateral preoptic area, and the anterior hypothalamic area led to inhibition or enhancement of firing rates of tuberomammillary neurons (15).

PHYSIOLOGIC ROLES OF HISTAMINERGIC NEURONS

In spite of many different suggestions mainly derived from the observations of responses to locally applied histamine, only a few physiologic roles of histaminergic neurons appear relatively well documented.

Arousal

Our initial proposal in 1977 (81) that histaminergic neurons play a critical role in arousal has been confirmed by data from a variety of experiments mainly performed by Lin and Jouvet in cats (33) and Monti in rats (2). In agreement with this concept, ablation of these neurons and inhibition of histamine synthesis, release, or action by the H_1 receptor decrease wakefulness and increase deep slow-wave sleep; conversely, inhibition of histamine methylation or facilitation of histamine release by H_3 receptor blockade increase arousal (49).

The arousing effect of histamine may result from H_1 and H_2-receptor–mediated depolarization of thalamic relay neurons that induces a shift of their activity from burst firing (predominating in deep sleep during which they are poorly responsive to sensory inputs) to single spike activity (predominating in arousal during which sensory information is more faithfully relayed) (31). Arousal may also result from H_1-receptor–mediated excitation of neocortical pyramidal neurons by the same mechanism as in thalamus, that is reduction of a background leakage potassium current (28). Finally, arousal may occur indirectly by H_1-receptor mediated excitation of ascending cholinergic neurons within the nucleus basilis or mesopontine tegmentum (32, 33), which also induces cortical activation.

All these cellular actions of histamine, together with observations that tuberomammillary neuron firing is maximal during wakefulness, suggest that histaminergic systems make an important contribution to the control of arousal. The circadian changes in histaminergic neuron activity seem to be directed by two major neuronal inputs arising from the anterior hypothalamus. The first ones are slow-wave sleep–activated inhibitory GABA- and galanin-containing neurons arising from the ventrolateral preoptic area (12,13); in contrast, neurons releasing the neuropeptide orexin that emanate from the lateral hypothalamus appear to exert opposite actions because disruption of the orexin gene is associated with narcolepsy in dogs and knockout mice (14,82). Other monoaminergic neurons participating in control of sleep and wakefulness states as well as GABA/galanin ventrolateral preoptic neurons also receive inputs from orexin neurons, which are, themselves, likely influenced by photic signals from the suprachiasmatic nucleus. In turn, neurons from the suprachiasmatic nucleus and the preoptic area seem to be influenced in a complex manner by histaminergic inputs (83,84). Hence a complex neuronal network in the hypothalamus with reciprocal influences involving histaminergic neurons seems to control wakefulness.

The major part played by the H_1 receptor in these processes, confirmed in mutant mice lacking this receptor (85), accounts for the sedating effects of the first generation of "antihistamines," that is, antagonists that easily enter the brain and are still ingredients of over-the-counter sleeping pills (86). It may also account for the sedative side effects of many antipsychotic or antidepressant drugs that are potent H_1 antagonists.

Cognitive Functions

The idea that histaminergic neurons may improve cognitive performance is consistent with projections of these neurons to brain areas such as the prefrontal and cingulate cortices or hippocampus, their excitatory influences therein, and their positive role in wakefulness.

Ciproxifan, a potent and selective H_3-receptor antagonist (or inverse agonist), which strongly enhances histamine turnover in brain, improved attentional performances in the rat five-choice test under conditions similar to those of drugs enhancing cholinergic transmissions (49). Various H_3 antagonists facilitate various forms of learning. They improve short-term social memory in rats (87), reverse the scopolamine- or senescence-induced learning deficit in a passive avoidance test in mice (88), and facilitate retention in a footshock avoidance test in mice (89).

Generally, H_3 agonists exert opposite effects, and the effects of H_3-antagonists are reversed by H_1 antagonists, a finding that suggests that these effects are attributable to enhanced histamine release. In contrast to the large body of experiments indicating a "procognitive" role of tuberomammillary neurons, Huston and coworkers repeatedly showed that excitotoxic lesions aimed at these neurons ablation result in improvement of learning in a variety of tests (e.g., ref. 90). The discrepancy with data from pharmacologic approaches may result from the difficulty to achieve selective histamine neuron ablation.

Control of Pituitary Hormone Secretion

Histamine affects secretion of several pituitary hormones (2,91). Magnocellular neurons of the supraoptic and para-

ventrical nuclei are typically excited, an essentially H_1-receptor–mediated response resulting in enhanced blood levels of vasopressin and oxytocin. Histaminergic neurons are activated during dehydration, parturition, and lactation, and histamine release onto magnocellular neurons participates in the control of these physiologic processes by the neurohypophysial hormones (92,93).

Histaminergic neurons may also participate in the hormonal responses to stress. In agreement with this concept, they are activated during various forms of stress and heavily project to hypothalamic or limbic brain areas (e.g., the amygdala or bed nucleus of the stria terminalis) involved in these responses. Various pharmacologic studies have shown the participation of endogenous histamine by H_1- and H_2-receptor stimulation in the adrenocorticotropic hormone–corticosterone, prolactin, or renin responses to stressful stimuli such as restraint, endotoxin, or dehydration (94). Many histaminergic neurons contain estrogen receptors, project to luteinizing hormone–releasing hormone neurons in preoptic and infundibular regions, and may constitute, by H_1-receptor stimulation, an important relay in the estradiol-induced preovulatory luteinizing hormone surge (95).

Satiation

Weight gain is often experienced by patients receiving H_1 antihistamines as well as by patients taking antipsychotics or antidepressants displaying potent H_1-receptor antagonist properties. These effects reflect the inhibitory role of endogenous histamine on food intake mediated by the H_1 receptor, namely, on the ventromedial nucleus (97). Histamine neurons projecting to the hypothalamus may be responsible for the food intake suppression induced by leptin (70).

Seizures

The anticonvulsant properties of endogenous histamine were initially suggested from the occurrence of seizures in patients with epilepsy, particularly children, after administration of high doses of H_1-receptor antagonists crossing the blood–brain barrier, even those agents devoid of anticholinergic activity (86). These drugs, by completely occupying the H_1 receptor, as assessed by positron emission tomography studies (30), could block the histamine-induced reduction of a background-leakage K^+ current.

Drug-induced changes in histamine synthesis, release or metabolism confirmed the role of the endogenous amine acting through the H_1 receptor in preventing seizure activity elicited by pentetrazol, transcranial electrical stimulation, or amygdaloid kindling . Acquired amygdaloid kindling susceptibility appears associated with reduced histamine synthesis in limbic brain areas (97). In addition, kainic acid–induced limbic seizures are accompanied by up-regulation of the H_1-receptor mRNA in striatum and dentate gyrus, a finding consistent with a regulatory role of this receptor in seizure activity (98).

Nociception

The antinociceptive effects of histidine loads, H_3-receptor antagonists, and histamine *N*-methyltransferase inhibitors, as well as opposite effects of histamine synthesis inhibitors or H_3 agonists, support the idea that brain histamine inhibits nociceptive responses such as the mouse hot plate jump (99). In contrast, peripherally acting H_3-receptor agonists prevent nociceptive responses such as mouse abdominal constriction by inhibiting sensory C-fiber activity (100).

ROLE OF HISTAMINERGIC NEURONS IN NEUROPSYCHIATRIC DISEASES

Among the various approaches that tend to establish the implication of other neuronal systems in neuropsychiatric diseases, so far only a few have been applied to histamine.

Histamine, Schizophrenia, and Antipsychotic Drug Actions

Overdose of various classic H_1 antagonists was repeatedly reported to result in toxic psychoses with hallucinations resembling schizophrenia, and the hallucinogenic potential of these drugs has even led to abuse (86). Conversely, metamphetamine, a drug with hallucinogenic potential and to which patients with schizophrenia seem hyperresponsive, releases histamine in rodent brain areas, an indirect effect mediated by stimulation of D2 and not D3 dopamine receptors (77,78). Even more, endogenous dopamine appears to exert a tonic stimulation of histamine neurons because typical neuroleptics, such as haloperidol, decrease their activity. In contrast, atypical neuroleptics, such as clozapine, enhance histamine turnover, an effect related to 5-HT_2 receptor blockade and possibly underlying their procognitive properties (77). The locomotor activation elicited in rodents by amphetamine and other dopaminergic agonists is attenuated by H_3-receptor blockade (101). Repeated amphetamine administration to rodents that results in behavioral sensitization to dopamine agonists, a cardinal feature of schizophrenia, is accompanied by enhanced histamine release, a finding that presumably reflects an enhanced tonic dopaminergic influence on histaminergic neurons (77,78). In one comprehensive study, an enhanced level of τ-methylhistamine, the major histamine metabolite, was detected in the cerebrospinal fluid of patients with schizophrenia, who were either treated or untreated with neuroleptic agents (102).

In several open studies, famotidine, an H_2 antagonist, was found to improve schizophrenia in patients, a finding

that remains to be confirmed in control studies. A previous claim of association between polymorphisms of the H_3-receptor gene and schizophrenia could not be confirmed (103).

These various observations, although not readily forming a coherent picture, suggest that histaminergic neuron activity is enhanced in patients with schizophrenia, and blockade of H_1 or H_3 receptors could be useful in the treatment of this disease.

Histamine and Alzheimer's Disease

Neuropathologic studies have documented a deficit in histaminergic neurotransmission in Alzheimer's disease. In some, but not all, cortical areas (e.g., the frontal or temporal cortex) of brains affected by Alzheimer's disease, there is a decrease of histamine and histidine decarboxylase levels that may reach up to approximately 50% (104), and the expression of the *hdc* gene in neurons of the tuberomammillary nucleus is also reduced (S. Trottier, personal communication). Decreased histaminergic input may affect cholinergic neuron activity in the nucleus basilis (32) and acetylcholine release in cortical areas.

If one takes into account an additional direct positive influence of histamine on attention and memory, this indicates that enhancing histaminergic neurotransmission may constitute a novel symptomatic therapeutic approach to Alzheimer's disease. The drug tacrine was even more potent in inhibiting histamine-*N*-methyltransferase, the main histamine-metabolizing enzyme, than acetylcholinesterase (105).

Histamine and Other Neuropsychiatric Disorders

Anxiety may be increased by endogenous histamine acting at the H_1 receptor. In agreement with this concept, H_1-receptor knockout mice display significantly less anxiety in the elevated maze test (106). However, the utility of H_1-receptor antagonists in anxiety disorders is not established.

Patients with *attention-deficit disorders* may benefit from enhanced histamine release, as suggested by the therapeutic effect of amphetamine in children and the attention-enhancing effects of an H_3-receptor antagonist in the rat (49). *Antidepressant-like effects* in the mouse forced swim test result from enhanced histamine release and H_1-receptor activation (107).

CONCLUSION

This chapter describes how our knowledge of the molecular neurobiology of cerebral histaminergic systems and their implications in physiologic functions, such as arousal or emotional regulations, have progressed over the years. In contrast, little is known, so far, about their possible implications in neuropsychiatric diseases and the therapeutic utility of psychotropic drugs to affect their activity. H_3-receptor antagonists (or inverse agonists) that markedly enhance brain histamine release are currently undergoing clinical trials. It seems likely that the next edition of this book will see their place in therapeutics established.

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near the JG-1 point, the two end members for mixing are required to be quite old. A possible example which would satisfy available constraints is the mixing of a magma derived from S_1-type source 4,000 Myr old, and 3,000 Myr old crust with L_3-type REE patterns. However, such old materials cannot be expected in Japanese Islands.

Another way to reach the ε_{Ce} and ε_{Nd} values of JG-1 is to have a considerable isotope growth in the L-type source which is derived from an S-type primary source. (A production of L type from S type can be realized by a small degree of partial melting of the S type source[19].) As discussed before, isotope growth in L-type material follows a trend of almost constant slope in the ε_{Ce}–ε_{Nd} diagram (see Fig. 3). If we assume that the L-type source material is derived from an S-type (for example, S_4) primary source, isotope growth is obtained as shown by the arrow in Fig. 5. In this case S_1-type material cannot be a primary source: this is because the L-type growth line passing through JG-1 does not cross the S_1 growth line at a geologically plausible time. For the petrogenesis of JG-1, of course, crustal contamination cannot be ruled out, but the effect by the contamination might be a secondary contribution, if present at all.

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Conclusion

Within the Nd-Sm isotope diagram there is a remarkably well-defined 'mantle array', which has provided tremendous insight into several important geochemical and geological questions. It is not yet clear whether there is a similar clearly defined mantle array in the Ce-Nd isotope system or not. Further improvement of analytical techniques, and accumulation of more data on cerium isotopes should enable us to investigate the evolution and the scale of mantle heterogeneities in more detail. In contrast to the present status of mantle Ce-Nd data, the Ce-Nd array for rocks of the continental crust is very well-defined. This Ce-Nd array is a very distinctive characteristic of continental crustal materials. La-Ce isotope systematics may make a large contribution to studies on crustal development.

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Highly potent and selective ligands for histamine H_3-receptors

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New drugs selective for histamine H_3-receptors can be used to establish that these receptors are involved in the feedback control of histamine synthesis and release, and to demonstrate their distribution in the brain and peripheral tissues. These drugs provide new tools for affecting physiological and possibly pathological conditions in which histamine is involved.

SINCE the discovery of histamine by Sir Henry Dale at the beginning of this century, the multiple functions of this amine as a chemical messenger in cell-to-cell communication have been progressively revealed. Histamine is stored in, and released from, various cellular populations including immunoglobulin E (IgE) receptor-bearing cells (mast cells and basophils)[1], endocrine cells[2] and neurons in the central[3] or peripheral nervous system[4]. An even larger variety of cell types including smooth muscles, neurons, endocrine or exocrine glands, blood cells and cells of the immune system, respond to histamine by increasing the intracellular levels of signals generated by either the phosphatidylinositol cycle[5] or adenylate cyclase[6]. Each of these two intracellular responses is mediated by a pharmacologically distinct subclass of receptors, the H_1- and H_2-receptors respectively[7].

More recently it was observed that histamine inhibits its own synthesis and release in brain slices by a negative feedback process operating at the level of histaminergic nerve-endings[8–10]. It was suggested that these actions of histamine are mediated by a novel subclass of receptors (H_3) because they are not blocked by H_1-receptor antagonists and, although some H_2-receptor antagonists were effective, their potencies differed from those found in reference H_2-receptor-mediated responses. No selective agonist or antagonist of histamine could be identified at putative H_3-receptors. Because a receptor subclass is ultimately defined by the use of highly selective agents and its function assessed by testing the agents in the living animal, we have undertaken to identify or design suitable compounds. We now describe the actions of (*R*)α-methylhistamine (α-MeHA), a chiral agonist, and thioperamide, an antagonist derived from imidazolylpiperidine, which both display high selectivity and potency at nanomolar concentrations *in vitro*. These drugs modify antagonistically histamine synthesis and/or release from brain slices and also cerebral and peripheral tissues in the living rat. In addition a partial agonist at H_3-receptors is described. Finally we show that [^{3}H]-(*R*)α-MeHA is a useful probe for

Table 1 Potencies of various imidazole derivatives at the three subclasses of histamine receptors

			H_3-Receptor			
gents	H_1 Receptor	H_2-Receptor	Inhibition of [^{3}H]histamine release	Inhibition of [^{3}H]histamine synthesis	Inhibition of [^{3}H]-(R)α-MeHA binding: Rat brain	Guinea-pig lung
gonists (relative potencies)						
Histamine	100	100	100	100	100	100
(R)α-MeHA	0.49*	1.02‡	1,550	1,130	1,060	813
(S)α-MeHA	0.49*	1.74‡	13		11	52
4-[2-(1-Pyrrolidinyl)ethyl]-imidazole	Antagonist* (K_i = 5 μM)	0.1‡ (30% max.)	7 (56% max.)		5	6
ntagonist (K_i values)						
Thioperamide	>100 μM†	>10 μM§	4.3 nM	31 nM	2.1 nM	2.0 nM

The relative potencies were calculated as the ratio (EC_{50} (or K_i in binding experiments) of histamine)/(EC_{50} (or K_i) of agonist) × 100. The EC_{50} nd K_i were determined from data shown in Figs 1 and 2. The relative potency of (R)α-MeHA in the inhibition of [^{3}H](R)α-MeHA binding in at brain was calculated from its K_D. The K_i of thioperamide in relation to the inhibition of [^{3}H]histamine release is the mean value of its various determinations described in Fig. 1 legend.

* Tested for stimulating contraction of the isolated guinea-pig ileum. Data from refs 44 and 45.
† Tested for competition with [^{3}H]mepyramine binding in membranes of guinea-pig cerebellum.
‡ Tested for rate stimulation in the spontaneously beating isolated guinea-pig right atrium. Data from ref. 44.
§ Tested for inhibition of cyclic AMP accumulation in slices from guinea-pig hippocampus.

he radioassay and autoradiographic visualization of H_3-recep-ors

A chiral agonist

Histamine is an achiral molecule which presumably adopts different preferred conformations when interacting productively with its various receptor subclasses. It appears to act at both H_1- and H_2-receptors as the N^τ-tautomer of the monocation, possibly adopting the *trans* conformation[11]. Analysis of the chemical properties of selective H_1 or H_2-receptor agonists have provided further insights into the molecular requirements of the corresponding receptor subclasses[11]. But none of these compounds is able to mimic the actions of histamine at H_3-receptors and some of them even behave as antagonists[8-10]. In addition a series of chiral histamine derivatives with branched side chains were used to elucidate the stereochemical requirements of the various receptor subclasses[12]. With these compounds no stereo selectivity and limited stereoselectivity were found at H_1- and H_2-receptors respectively, whereas a high degree of stereoselectivity, the reverse of that observed at H_2-receptors, was recently found for two compounds (N^α methyl-α-chloromethylhistamine and α, N^α-dimethylhistamine) at H_3-receptors. However both active enantiomers behaved as weak agonists, their potency at H_3-autoreceptors being only 1-4% that of histamine[13].

In contrast, by systematically studying chiral analogues with a single substitution of the side chain in an attempt to reduce the conformational freedom of the histamine molecule and thereby increase its specificity without losing its activity, we have identified α-MeHA as a compound that fulfills these requirements. In the H_3-receptor assay system in which depolarization-induced release of [^{3}H]histamine from brain slices is evaluated[8], both (R) and (S)α-MeHA progressively inhibited the release by up to about 60% that is, to the same maximal extent as exogenous histamine itself (Fig. 1a). This suggests that both enantiomers behaved as full H_3-receptor agonists, but the (R) isomer exerted this effect at nanomolar concentrations, being approximately 100 times as potent as the (S) isomer and 15 times as potent as histamine itself. This is in agreement with previous observations showing that enantiomers corresponding to S-configurated L-histidine are highly preferred at H_3-receptors whereas enantiomers corresponding to D-histidine are slightly more potent at H_2-receptors, no difference being observed at H_1-receptors[13]. As the (R)α-MeHA potencies relative to histamine are only 0.5% and 1% at reference H_1- and H_2-receptor systems respectively (Table 1), it can be regarded as a highly potent and selective H_3-receptor agonist. Because H_3-receptors are more sensitive to histamine and agonists than H_1- or H_2-receptors, (R) α-MeHA might even be regarded as more selective if one considers its pD_2 value (negative logarithm of the EC_{50}), which is, 4.54, 3.96 and 8.40 at the reference H_1-, H_2- and H_3-receptor systems respectively. This means that stimulation of H_3-receptors by (R)α-MeHA is expected to occur at concentrations ~10,000 times lower than those required for H_1- or H_2-receptor stimulation.

The H_3-autoreceptors seem to control not only release of [^{3}H]histamine from, but also its synthesis in, depolarized cerebral histaminergic neurons[10,14]. Consistently, (R)αMeHA potently inhibited the K^+-induced stimulation of [^{3}H] histamine formation in slices of rat cerebral cortex (Fig. 1d).

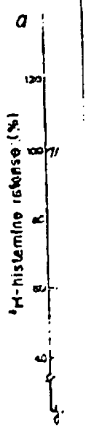
That these effects of (R)α-MeHA are mediated by H_3-receptors was confirmed by their blockade by impromidine[8] (not shown) and the novel antagonist thioperamide (see below).

A competitive antagonist

In seeking a specific antagonist of histamine at cerebral H_3-receptors able to cross the 'blood–brain barrier', the possibility of using known H_1- or H_2-receptor ligands as chemical starting point was first considered. But the lipophilic H_1-receptor antagonists tested displayed negligible affinity[9]. In contrast several agonists at H_1-receptors, such as betahistine[15], or at H_2-receptors, such as impromidine, as well as H_2-receptor antagonists, such as burimamide, displayed significant antagonist activity[8]. But these are generally highly hydrophilic and/or positively charged molecules with little ability to enter the brain from the circulation.

Hence we took a completely different approach in which the histamine molecule was used as the chemical starting point and analogues were synthesized in which the ethylamine side chain was extended and its nitrogen atom included in a piperidine ring. In all cases this resulted in a loss of agonist activity. The affinity of compounds for H_3-receptors was progressively optimized by varying the nature of the piperidine-substituting groups and thioperamide (MR 12842, *N*-cyclobexy-4-(imidazol-4-yl)-1-piperidinecarbothioamide), was finally selected.

When thioperamide was opposed at a fixed concentration to histamine, the concentration-response curve to the amine was shifted to the right without change of the maximal response, leading[16] to an apparent K_i of 7.6 nM (Fig. 1a). Similar apparent dissociation constants for thioperamide were derived[17] from the analysis of the progressive reversal of the inhibition of [^{3}H]histamine release exerted by either exogenous histamine or



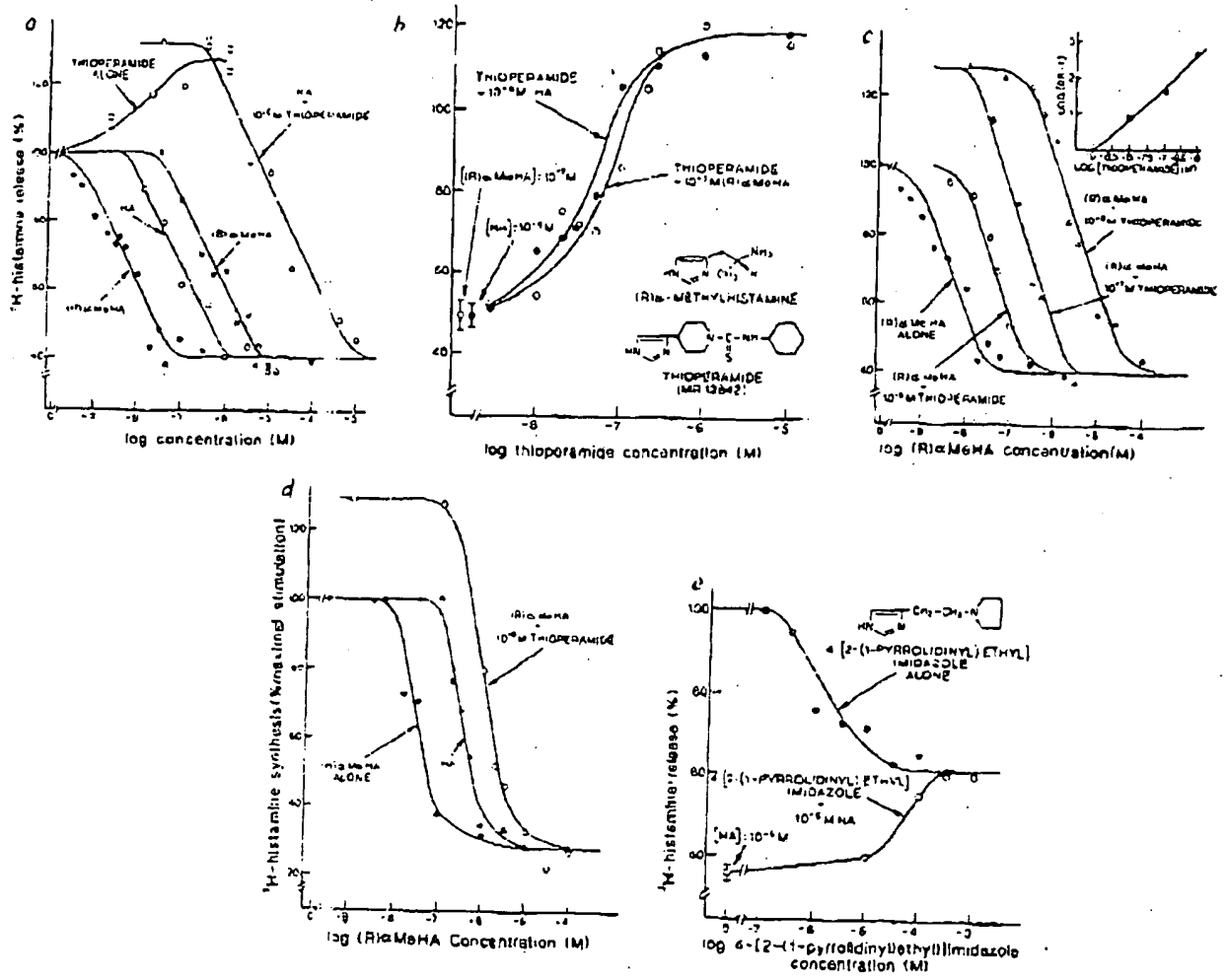


Fig. 1 Effects of various imidazole derivatives on the K^+-induced release of [^{3}H]histamine from, and stimulation of [^{3}H]histamine synthesis in, slices of rat cerebral cortex. *a*, Release of [^{3}H]histamine was inhibited by histamine (HA) (○, left-hand slope), (*R*)α-MeHA (●) and (*S*)α-MeHA (×) with EC_{50} values (nM) of 62 ± 14, 4.0 ± 0.8 and 470 ± 160 respectively. Maximal inhibitions were 62 ± 2% in all cases. Thioperamide alone (□) maximally increased [^{3}H]histamine release by 24 ± 3% (half-maximal concentration: 4.1 ± 3.5 nM). In the presence of 1 μM thioperamide the EC_{50} of histamine became 8.2 ± 2.6 μM, leading[19] to a K_i of 7.6 nM for the antagonist (○, right hand slope). *b*, The IC_{50} values (nM) of thioperamide were 48 ± 9 and 74 ± 17 when opposed to histamine (HA, ●) and (*R*)α-MeHA (○) respectively, leading[17] to K_i values (nM) of 5.6 ± 1.0 and 2.9 ± 0.5. *c*, Thioperamide at fixed concentrations was opposed to (*R*)α-MeHA in increasing concentrations (release experiments) (●, (*R*)α-MeHA alone; and with ○, 10^{-8} M; ▲, 10^{-7} M and △, 10^{-6} M thioperamide). Inset, Schild plot of data in which dose ratios (DR) were determined from EC_{50} values of (*R*)α-MeHA. The slope and 95% confidence limits for the regression of log (DR − 1) on log [thioperamide] was 0.91 (0.82–1.01), the coefficient of correlation was 0.995 and the pA_2 8.96. *d*, The K^+-induced stimulation of [^{3}H]histamine synthesis was inhibited (−71 ± 4%) by histamine and (*R*)α-MeHA with EC_{50} values (nM) of 360 ± 30 and 30 ± 7 respectively. Symbols: ●, (*R*)α-MeHA alone; ▲, histamine; ○ (*R*)α-MeHA with 10^{-6}M thioperamide. *e*, 4-[2-(1-pyrrolidinyl)ethyl]imidazole alone (●) inhibited the K^+-evoked release of [^{3}H]histamine by 36 ± 2% (as compared to 64 ± 2% for histamine) and with an EC_{50} of 0.9 ± 0.3 μM. When opposed to histamine (○) its IC_{50} was 38 ± 7 μM and a plateau corresponding to 39 ± 1% inhibition of release was reached. From this an intrinsic activity of 56% that of histamine and a K_i of 2.2 ± 0.4 μM were derived.
Methods. In *a*, *b*, *c* and *e* the K^+-evoked release of [^{3}H]histamine was evaluated as described[6]. After preincubation with L-[^{3}H]histidine (Amersham), slices were incubated for 2 min with 2 or 30 mM K^+. Drugs were added 5 min before the start of incubations, at the end of which [^{3}H]histamine was assayed[17] in tissue and medium. The spontaneous efflux of [^{3}H]histamine into the medium (2 mM K^+) represented 5.0 ± 0.2% of the total (tissue plus medium) and was not altered by any of the added agents. In the absence of added agents, the [^{3}H]histamine release evoked by 30 mM K^+ (expressed as percentage of total [^{3}H]histamine released in the medium over spontaneous efflux) represented 14.2 ± 0.6%. Results are expressed as percentages of this value. Means from 2–5 different experiments with at least triplicate determinations. In *d*, the K^+-induced stimulation of [^{3}H]histamine synthesis was evaluated in slices prepared as described[6]. Aliquots of the slice suspension (~4 mg protein) were incubated for 30 min at 37 °C under a stream of O_2/CO_2 (95:5) in the presence of 0.55 μM [^{3}H]histidine and 2 or 30 mM K^+. Drugs were added at the beginning of incubations which were stopped by sonication of the total samples (tissue plus medium) and addition of $HClO_4$ (0.4 M final concentration). The [^{3}H]histamine was isolated on two successive Amberlite CG 50 columns[18] with a 77 ± 3% recovery, contamination by [^{3}H]histidine being <0.001%. In the absence of added agents [^{3}H]histamine synthesis represented (d.p.m. per mg protein) 2,055 ± 124 (2 mM K^+) and 3,953 ± 248 (30 mM K^+). The difference between these two values was taken as the K^+-induced stimulation and represented 1,898 ± 277 d.p.m. per mg protein (92% increase). Results are expressed as percentages of this value and correspond to 2–7 experiments with at least triplicate determinations. For determination of EC_{50} and IC_{50} values, the total curves were analyzed with an iterative computer least-squares method[20]. Values of K_i were determined neglecting the influence of endogenous histamine.

R)α-MeHA in fixed concentration (Fig. 1*b*). Also, Schild plot nalysis of the progressive shift to the right of the concentration-sponse curve to (*R*)α-MeHA elicited by thioperamide, com-atible with a competitive antagonism by this compound, led a pA_2 of 8.96, corresponding to a K_i of 1.1 nM (Fig. 1*c*). In ddition, thioperamide alone elicited in a concentration-depen-dent manner a small but consistent increase (~25%) in [^{3}H]his-tamine release with half-maximal effect at 4.1 nM (Fig. 1*a*). This facilitatory effect was previously observed with other H_3-receptor antagonists and attributed to antagonism of endogenous histamine released by the depolarizing stimulus and exerting its normal negative feedback influence on the release

process. All these consistent data indicate that thioperamide competitively blocks H_3-autoreceptors regulating [^{3}H]histamine release with a mean apparent K_i of 4 nM, ignoring the influence of endogenous histamine (Table 1). The competitive nature of thioperamide antagonism was confirmed in radioligand binding studies.

Finally, thioperamide also antagonized in a surmountable manner the (*R*)α-MeHA-induced inhibition of [^{3}H]-histamine synthesis with a K_i of 31 nM (Fig. 1*d*). This value, slightly higher than that found in the release studies, may conceivably result from a stronger influence of endogenous histamine, reaching higher extracellular concentrations after the longer-lasting depolarizations used in synthesis studies. Thioperamide has negligible affinity for H_1- and H_2-receptors (Table 1) as well as for α- or β-noradrenaline[12,19] ($K_i > 1$ μM), muscarinic[20] ($K_i >$ 10 μM), opiate[21], dopamine[22] or serotonin[23,24] ($K_i > 0.1$ mM) receptors. This indicates that thioperamide is a potent and selective H_3-receptor antagonist.

A partial agonist

Although alkylation of the histamine side-chain terminal nitrogen with bulky groups leads to a loss of H_3-receptor agonist activity[25], its introduction into a pyrrolidine nucleus led to a compound which was still able to mimic histamine partially in inhibiting the [^{3}H]amine release (Fig. 1*e*). However, the relative potency was reduced to 7% and, interestingly, the maximal response was only 56% that of histamine, suggesting that this compound may represent a partial agonist. This was confirmed by opposing it to histamine, whose maximal response was reduced to the same level, leading to a K_i of 2.2 μM for 4-[2-(1-pyrrolidinyl)ethyl] imidazole when considered as a pure antagonist.

Action *in vivo*

The availability of potent agents acting selectively at H_3-receptors made it possible for the first time to assess the physiological function of these receptors in the control of histamine synthesis and release in brain and peripheral organs of living animals. In rats treated with thioperamide (5 mg per kg, intraperitoneally (i.p.)) the steady state histamine level in a subcellular fraction from cerebral cortex enriched in nerve endings was reduced by 30% after 30 min (Table 2). This presumably reflected an increased rate of [^{3}H]histamine release because the H_3-receptor antagonist also accelerated by about twofold the amine depletion observed after irreversible inhibition of its synthesis by α-fluoromethylhistidine[26]. The reversal of this effect by the agonist (*R*)α-MeHA (20 mg per kg, i.p.) is consistent with the idea that it is H_3-receptor-mediated (Table 2). That (*R*)α-MeHA alone modestly increased the steady state level of histamine but completely prevented the histamine depletion elicited by inhibition of its synthesis presumably reflects a marked reduction of histamine release *in vivo*. This effect of the agonist was stereo selective, as shown by the non-significant change in α-fluoromethylhistidine-induced depletion 45 min after administration of (*S*)α-MeHA which, *in vitro*, is 100-fold less potent than the (*R*)isomer.

Such opposite effects of the agonist and the antagonist were also found when [^{3}H]histamine formation in cerebral cortex was evaluated after administration of its ^{3}H-labelled precursor. Hence (*R*)α-MeHA (10 mg per kg, intravenously (i.v.)) significantly decreased [^{3}H]histamine formation by about 30% and this was reversed by thioperamide (10 mg per kg, i.p.) which, alone or in combination with (*R*)α-MeHA, markedly enhanced the ^{3}H-labelled amine formation (Table 3). A qualitatively similar effect occurred in the hypothalamus, where histamine perikarya (and also terminals) are located[3,27,28]. Hence it seems that both α-MeHA and thioperamide cross the blood-brain barrier at reasonable dosages and that brain histamine neurons activity can be modulated bi-directionally by occupation of

Table 2 Effects of α-methylhistamine and thioperamide on steady-state level and metabolism of histamine in rat cerebral cortex

Treatment	Time after treatment (min)	Histamine level (ng per g protein)	(% change)
Control		547 ± 24	
Thioperamide	30	381 ± 21†	−30
(*R*)α-MeHA	30	626 ± 47	+15
(*S*)α-fluoromethylhistidine:			
alone	30	438 ± 30*	−20
+thioperamide	30	313 ± 111‡	−43
+(*R*)α-MeHA	30	653 ± 38*‡	+19
+thioperamide +(*R*)α-MeHA	30	602 ± 56‡	+10
Control		532 ± 26	
(*S*)α-Fluoromethylhistidine:			
alone	45	305 ± 29†	−43
+(*R*)α-MeHA	45	605 ± 23‡	+14
+(*S*)α-MeHA	45	367 ± 17†	−31

Male Wistar-rats (80–110 g) received the various drugs i.p. and were killed by decapitation 30 or 45 min later. Doses of (*R*)- and (*S*)α-MeHA hydrochloride were given at 20 mg per kg (expressed as the base), thioperamide at 5 mg per kg and (*S*)α-fluoromethylhistidine, an irreversible inhibitor of L-histidine decarboxylase[26] (from Dr J. Kollonitsch, MSD Research Laboratories) at 100 mg per kg. The cerebral cortex was dissected out in the cold, homogenized in 10 vols of ice-cold 0.32 M sucrose using a Teflon-glass Potter-Elvehjem homogenizer (clearance 0.10–0.15 mm). A crude P_2 synaptosomal fraction was prepared by two centrifugations (10,000 and 400,000*g* min respectively), the last pellet being resuspended in phosphate buffer (K_2/K 20 mM, *p*H 7.6). Endogenous histamine level in this fraction was estimated with a radioenzymatic assay[43] using [^{3}H]*S*-adenosyl methionine (76 Ci mmol^{-1}; Amersham) and a purified preparation of rat kidney histamine *N*-methyltransferase. The inactivation of L-histidine decarboxylase in the synaptosomal fraction from (*S*)α-fluoromethylhistidine-treated rats was controlled using a radioassay[43]. Inhibition was nearly total (90 ± 2%) from 15 min to 240 min. Histamine depletion in rats receiving (*S*)α-fluoromethylhistidine alone had an initial half-life of about 40 min and the maximal decrease was 62% after 120 min (not shown), consistent with previous reports[3,26]. Values are means ± s.e.m. from groups of 8–12 treated rats and 8–21 vehicle-treated rats (controls) respectively.

* $P < 0.006$ and † $P < 0.0001$ as compared with controls. ‡ $P < 0.02$ as compared with (*S*)α-fluoromethylhistidine alone.

Table 3 Effects of (*R*)α-methylhistamine and thioperamide on [^{3}H]histamine formation in various rat tissues

Tissues	Controls	[^{3}H]histamine levels (d.p.m. g^{-1} × 10^{-2}) Treated with (*R*)α-MeHA	Thioperamide	(*R*)α-MeHA + thioperamide
Cerebral cortex	32 ± 2	22 ± 2† (−29%)	75 ± 8† (+138%)	63 ± 5† (+99%)
Hypothalamus	103 ± 6	88 ± 5 (−15%)	253 ± 26† (+145%)	212 ± 22† (+105%)
Lung	51 ± 4	37 ± 3† (−29%)	96 ± 16† (+86%)	73 ± 12* (+42%)
Abdominal skin	144 ± 17	94 ± 13* (−34%)	109 ± 18	106 ± 33
Spleen	26 ± 3	18 ± 2* (−31%)	32 ± 6	25 ± 5
Colon	61 ± 3	50 ± 5	49 ± 8	46 ± 5

Groups of male Wistar rats (80–110 g) received 250 μCi of previously purified [^{3}H]L-histidine in the tail vein 10 min before being killed. The (*R*)α-MeHA was administered i.v. at 10 mg per kg together with [^{3}H]L-histidine, whereas thioperamide (10 mg per kg, i.p.) was administered 1 h before. The various tissues of each rat were rapidly dissected out and homogenized in 10 vol (w/v) of ice-cold 0.4 M $HClO_4$. The resulting homogenates were centrifuged (50,000*g* min) and [^{3}H]histamine was isolated from the supernatants by chromatography on two successive Amberlite CG-50 columns, then quantified by liquid scintillation spectrometry[29]. The recovery of [^{3}H]histamine was 73% whereas contamination by [^{3}H]L-histidine was 0.016% and results were corrected accordingly. The various treatments did not significantly modify the total radioactivity in tissues. Values are means ± s.e.m. of measurements from 13–36 rats.

* $P < 0.04$; † $P < 0.005$ as compared with controls.

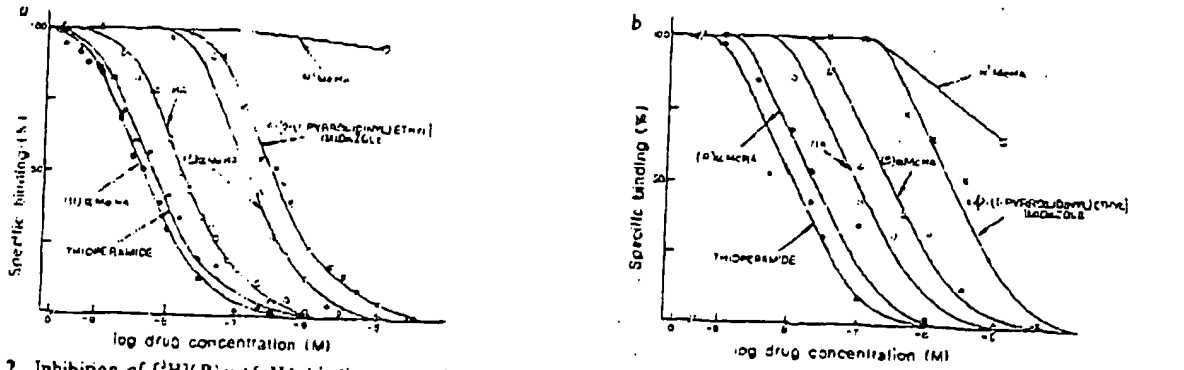


Fig. 2 Inhibition of [^{3}H](*R*)α MeHA binding to membranes of rat cerebral cortex (*a*) and guinea-pig lung parenchyma (*b*) by various imidazole derivatives. *a*, Values of K_i for the compounds were determined from their IC_{50} according to the equation[17] $K_i = IC_{50}/(1 + S/K_D)$ where *S* is the [^{3}H](*R*)α-MeHA concentration (1 nM) and K_D its dissociation constant (0.5 ± 0.2 nM) as determined from saturation kinetics at equilibrium. Pseudo Hill coefficients of these compounds did not significantly differ from unity. Values of K_i (nM) were 5.3 ± 0.3 (histamine ○), 1.3 ± 0.1 ((*R*)α-MeHA, ●), 50 ± 10 ((*S*)α-MeHA, △), 110 ± 10 (4-[2-(1-pyrrolidinyl)ethyl]imidazole, ×), 2.1 ± 0.3 (thioperamide, ▲), >3,000 (N^τ MeHA, the major histamine metabolite in brain, □), and are reported as such or as relative potencies for agonists in Table 1. The K_i of ten additional compounds (including impromidine, burimamide, cimetidine, tiotidine, ranitidine, mepyramine) were highly correlated ($r = 0.95$) with their K_i at H_3-receptors controlling [^{3}H] histamine release (J.-M.A., M.G., J.-L. Morgat, H.P., J. Roy, W.S. and J.-C.S., manuscript in preparation). *b*, Values of K_i, calculated as described in *a* and assuming the same K_D for [^{3}H](*R*)α-MeHA, were (nM) 26 ± 40 (histamine), 3.2 ± 2.8 ((*R*)α-MeHA), 50 ± 10 ((*S*)α-MeHA), 460 ± 120 (4-[2-(1-pyrrolidinyl)ethyl] imidazole, 2.0 ± 0.7 (thioperamide) and >2,000 (N^τMeHA). Symbols, as in *a*. In addition, K_i values for mepyramine and tiotidine were > 1 μM (not shown).
Methods. In *a*, a particulate fraction was obtained after homogenization of the cerebral cortex in 50 volumes (w/v) of ice-cold Na_2/K phosphate buffer 50 mM, *p*H 7.5 and two successive centrifugations (1,000*g* min and 200,000*g* min). After resuspension of the last pellet in fresh buffer, 0.9 ml aliquots of the suspension (0.4 mg protein) were incubated for 60 min at 25 °C with 1 nM of [2.5-^{3}H](*R*)α-MeHA (specific activity 20 Ci mmol^{-1}) and unlabelled substances in a final volume of 1 ml. After addition of 6 ml of ice-cold buffer, the mixture was filtered over vacuum onto Millipore AAWP filters which were then rinsed twice with 5 ml ice-cold buffer. Specific binding was defined as that inhibited by 1 μM thioperamide and represented 250 d.p.m. out of a total of 400 d.p.m. (62 ± 2%). Results are expressed as percentages of this value, each point representing the results from one to three different experiments with triplicate determinations. With all agents maximal inhibition did not exceed specific binding. In *b*, guinea-pigs (300–500 g) were killed and the heart and lungs removed together and placed on ice. The airways were carefully combed to separate parenchymal tissue from the bronchial tree. The lung parenchyma was homogenized in 10 volumes (w/v) of ice-cold phosphate buffer and submitted to two successive centrifugations (5,000 and 550,000*g* min respectively). In order to eliminate extensively endogenous histamine from the preparation, the pellet was washed six times by resuspension in fresh buffer and recentrifugation at 550,000*g* min. The final pellet was resuspended in fresh phosphate buffer and 0.9 ml aliquots (0.5 mg protein) were incubated as in *a*, except that [^{3}H](*R*)α-MeHA concentration was 2.0 nM and filters were Millipore SMWP. Radioactivity of the filters was counted in ACS scintillation fluid at 40% efficiency and 98% reproducibility. At least 2,000 disintegrations were counted over a background of 16 c.p.m. Specific binding, defined as in *a*, represented 50 d.p.m. out of a total of 160 d.p.m. (31 ± 5%). Results are expressed as percentages of this value, each point representing the results from one to three different experiments with at least triplicate determinations. With all agents maximal inhibition did not exceed specific binding.

H_3-receptors. Thioperamide seems to be the first drug known clearly to increase this activity.

Most interestingly, [^{3}H]histamine synthesis was modified in lung in the same manner as in brain (Table 3), suggesting that there are H_3-receptors there as well, a hypothesis confirmed by radioligand binding studies. Because a large part of lung histamine is located in mast cells found in abundance in the bronchial mucosa, in the alveolar septal connective tissue and in the pleura[29], it seems likely that these cells are endowed with H_3-receptors controlling the synthesis of the amine and, possibly, its release.

The effects of the H_3-receptor antagonist on [^{3}H]histamine formation were less clearcut in other peripheral tissues. For instance in skin, where mast cells of the connective tissue type are abundant[30], or in spleen, (*R*)α-MeHA also decreased [^{3}H]histamine formation by about 30% but the reversal by thioperamide was less clear because the H_3-receptor antagonist did not promote [^{3}H]histamine formation over control levels. In these tissues, although the presence of H_3-receptors remains to be confirmed by other approaches, it cannot be excluded that the modest effects of thioperamide are related to the absence of a tonic release of histamine under basal conditions.

Receptor assay and visualization

The high apparent affinity of (*R*)α-MeHA in functional studies suggested that the drug might constitute, when radiolabelled, a suitable probe for assay and visualization of H_3-receptors.

Indeed [^{3}H](*R*)α-MeHA was found to bind in a reversible and saturable manner to cerebral cortex membranes with a K_D of 0.5 nM (derived from either saturation kinetics or dissociation/association rates) and a maximal number of sites representing 30 ± 3 fmol per mg protein (J.-M.A., M.G., J.-L. Morgat, H.P., J. Roy, W.S. and J.-C.S., manuscript in preparation). In comparison H_1- and H_2-receptors appear significantly more abundant[31–33]. Sites labelled with [^{3}H](*R*)α-MeHA were pharmacologically identified as H_3-receptors by competition studies with a variety of compounds (Fig. 2*a*) whose potencies relative to histamine (for agonists) or K_i values (for antagonists) were in good agreement with corresponding values derived from functional studies (Table 1). Nevertheless, note that K_i values of histamine and agonists in binding studies were generally 5–10 times lower than their corresponding EC_{50} values in functional studies. This difference might correspond to differences in the ionic composition of media used in binding and functional studies or to the selective labelling of H_3-receptors in a discrete conformational state with high affinity for agonists.

In membranes of guinea-pig lung, specific binding sites could also be detected and unambiguously characterized as H_3-receptors (Fig. 2b and Table 1) in spite of their extremely low density (5 ± 2 fmol per mg protein), which confirms the presence of H_3-receptors outside the brain. In autoradiographic studies performed with [^{3}H](*R*)α-MeHA (Fig. 3), H_3-receptors were found to be fairly widespread in rat brain as previously shown in release studies[9]. Telencephalic areas such as the cerebral cortex (particularly in its most rostral part), striatum, hippocampus (molecular layer of the dentate gyrus), lateral septum, bed

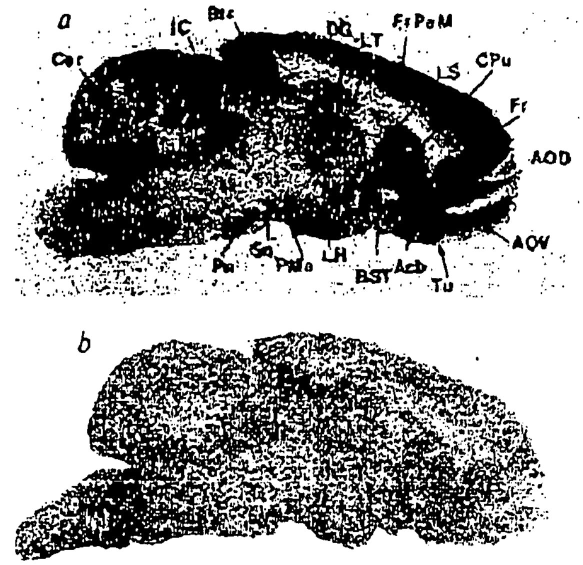


Fig. 3 Autoradiographic visualization of H_3-receptors in rat brain using [^{3}H](*R*)α-MeHA. Sagittal sections performed at level L: 1.4 mm of the stereotaxic atlas of Paxinos and Watson[50]. *a*, Total binding in the presence of 1 nM [^{3}H](*R*)α-MeHA; *b*, non-specific binding obtained by addition of 1 μM thioperamide in the incubation medium. Note the low and rather uniform distribution of autoradiographic grains in *b*. Similar pictures were obtained when thioperamide was replaced by 30 nM (*R*)α-MeHA whereas 30 nM (*S*)α-MeHA did not significantly modify the [^{3}H]ligand binding (not shown). Abbreviations: Acb, Accumbens nucleus; AOD, anterior olfactory nucleus dorsal; AOV, anterior olfactory nucleus, ventral, BST, bed nucleus of stria terminalis; Cer, Cerebellum; CPu, caudate putamen; DG, dentate gyrus; Fr, frontal cortex; FrPaM, frontoparietal motor cortex; IC, inferior colliculus; LH, lateral hypothalamic area; LS, lateral septum; LT, lateral thalamus; PMa, perimammillary area; Pn, pontine nuclei; Sn, substantia nigra; Str, striate cortex; Tu, olfactory tubercule.

Methods. Male Wistar rats (180–200 g) were killed by decapitation, their brains rapidly removed, dropped into cold freon (−40 °C monochlorodifluoromethane, Prestogaz R22) for a few minutes and kept frozen at −20 °C until used. Sagittal frozen sections (20 μM thick) were prepared in a Ames cryostat at −18 °C, thaw-mounted onto gelatin-coated glass slides and stored at −20 °C until needed. Incubations (45 min at 25 °C) were performed on slide-mounted tissue sections by dipping them into 1 nM[^{3}H](*R*)α-MeHA in 50 mM phosphate buffer pH 7.4 containing 0.2% gelatin. Non specific binding was obtained on immediately adjacent sections by adding 1 μM thioperamide in the incubation medium. The incubations were ended by dipping the slides briefly in drug-free ice-cold buffer and the tissue sections were rinsed sequentially in two 3-min phosphate buffer baths, then dipped into distilled water to remove any salts, immediately dried and apposed to [^{3}H]Ultrofilms (LKB) in X-ray cassettes for 12 weeks at 4 °C. Films were developed with Kodak D 19 (18 °C; 5 min), fixed in Kodak Unifix (10 min) and rinsed under running tap water (30 min).

nucleus of the stria terminalis, and olfactory nuclei, to which diffuse projections of the ascending histaminergic neurons have been described[3], showed the highest grain densities. In contrast the cerebellum (all layers); brainstem or mesencephalon (except a few areas, such as the substantia nigra), which contain a lower density of projections, showed fainter labelling. In the posterior hypothalamus a thin band of dense labelling was observed in the perimamillary area which is know to contain most histamine perikarya[3,27,28] suggesting the presence of H_3-receptors on the latter. However in the remainder of hypothalamus, in which levels of L-histidine decarboxylase and histamine are much higher than in telencephalon[3], a relatively low degree of labelling was observed. This may indicate that H_3-receptors density is not the same on all histamine neurons and/or that H_3-receptors are not restricted to the latter.

Conclusions and prospects

The potent and selective compounds that we describe here have already allowed us not only to confirm the existence of a third subclass of histamine receptors in brain but also to locate them and to elucidate their physiological involvement in the control of histamine synthesis and release in the living animal. In this respect the function of H_3-receptors seems to be analogous to that of other classes of presynaptic receptors mediating feedback regulation of several neurotransmitters[34–36]. Among these, α_2-adrenoreceptors seem negatively coupled to adenylate cyclase[37] and it would be of interest to assess whether H_3-receptors function similarly. The novel pharmacological tools, particularly the antagonist which appears to be the first agent able to elicit a marked facilitation of histaminergic transmissions in the central nervous system, should be useful, in behavioural and other studies, for defining further the functions of histamine systems. It has been suggested that the latter may be involved in the control of states of sleep and wakefulness[38–40], cerebral circulation[41], energy metabolism[42] and hypothalamic hormone secretion[43], but hitherto, verification of these hypotheses has been hindered largely by the lack of adequate pharmacological tools.

An application of the novel agents that is already important is the identification of H_3-receptors outside the central nervous system in tissues such as the lung in which they probably control mast-cell histamine formation and, possibly, release. Should this be the case, it might be of great practical importance for the understanding and treatment of allergic and inflammatory mechanisms, to establish whether other mediators, coexisting with histamine in mast cells, are also controlled by H_3-receptors.

Finally, using the novel agents, H_3-receptors with a pharmacology closely similar to those characterized in laboratory animals have been unambiguously identified in human brain (J.-M.A., M.G., J.-P. Chodkiewicz and J.-C.S., manuscript in preparation). This suggests that these, or similar agents, may find therapeutic applications.

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LETTERS TO NATURE

ravitational-wave bursts with memory id experimental prospects

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perimenters usually divide the gravitational waves which they e to detect into three classes: 'bursts' in which the wave field rises from zero, oscillates for only a few cycles and then urns to zero; 'periodic waves', and 'stochastic waves'[1]. There however, a fourth class, 'bursts with memory'[2–6] (BWM), in ich h_{ij}^{TT} rises from zero, oscillates for a few cycles, and then er a burst of duration Δt settles down into a non-zero final ue δh_{ij}^{TT}. Here we show that for any kind of detector the best / to search for a BWM is to integrate up the signal for an gration time $\bar{\tau} \simeq 1/f_{opt}$, where f_{opt} is the frequency at which detector has optimal amplitude sensitivity to ordinary bursts rsts without memory). In such a search the sensitivity to BWM h duration $\Delta t \lesssim 1/f_{opt}$ is independent of the burst duration Δt l is approximately equal to the sensitivity to ordinary bursts cycle long with frequency f_{opt} (see Fig. 1). It is possible, though highly probable, that BWM will be among the earliest kinds gravitational waves detected; therefore experimenters should e them into account when planning their search strategies and a analyses.

he reason that the optimum integration time is $\bar{\tau} \simeq 1/f_{opt}$ is ple: if a BWM barely rises above the noise in the data after te integration time $\bar{\tau}$, then one cannot know what its actual ation Δt was; one can only know that there was some net nge δh_{ij}^{TT} in the wave field during the time $\bar{\tau}$. This means , independently of Δt (for $\Delta t < \bar{\tau}$), the BWM will appear a BWM of duration $\bar{\tau}$, which in turn will be only marginally inguishable from an ordinary burst of one cycle length and ation $\bar{\tau}$. The optimal sensitivity to such an ordinary burst urs, by definition, at the frequency f_{opt}. Thus, the optimal itivity to a BWM must be for $\bar{\tau} \simeq 1/f_{opt}$ and must equal the inary burst sensitivity at frequency f_{opt}.

his argument is independent of the type of detector used: nant bar, laser interferometer on Earth or in space, monitor- of the Earth's seismic motions, skyhook, Doppler tracking pacecraft, or timing of pulsars. However, for each type of ctor there may be factors of order two to be gained, in ches for BWM, by careful planning of data analysis (optimal al processing) and by careful planning of the experimental p.

gure 1 shows the sensitivities (for unity signal-to-noise ratio) some present gravity-wave detectors and the limiting itivities of some detectors that have been proposed for future

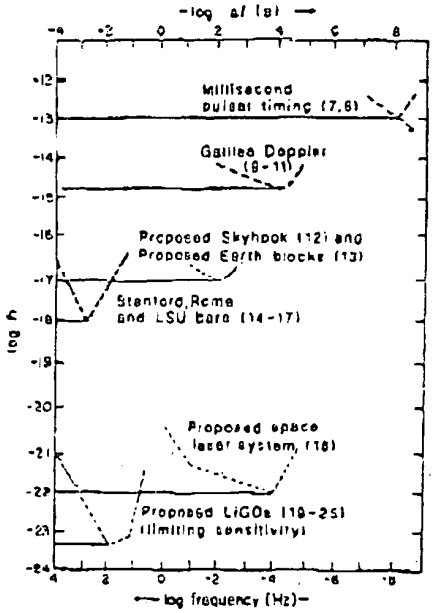

Fig. 1 Sensitivities of present (thick curves) and proposed (thin curves) gravitational-wave detectors. Dashed curves are sensitivities (dimensionless gravity-wave amplitude h) to ordinary bursts of one cycle duration, $\Delta t = 1/(\text{frequency})$. Solid curves are sensitivities to bursts with memory. Reference numbers are given in parentheses.

construction. The dashed curves are sensitivities to ordinary bursts; the solid curves, sensitivities (according to the above rule-of-thumb) to BWM. The thick curves are detectors now operating or due to operate in the near future. The thin curves are detectors proposed for construction and operation in the 1990s or (for laser system in space) in ~2,000. By 'sensitivity to ordinary bursts' (dashed curves) we mean sensitivity to a sinusoidal signal at the given frequency (bottom axis), which turns on abruptly, lasts for one cycle, then turns off abruptly. Details of the operating or proposed detectors will be found in the following references: search for fluctuations in the period of the millisecond pulsar relative to atomic clocks (primary frequency standards) (now underway)[7,8], Doppler tracking of the Galileo spacecraft (due to launch by NASA in late 1980s)[9–11], proposed Earth-orbiting skyhook[12], proposed multi-station monitoring of seismic vibrations of blocks of the Earth's crust with sizes 50 to 70 km[13], Stanford, Rome, and Louisiana State University cryogenically cooled, resonant aluminium bars (now operating)[14–17], proposed laser interferometer detector in space with 100 mW of laser power and with drag-free satellites at

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Neurochemical and Behavioral Effects of Ciproxifan, A Potent Histamine H_3-Receptor Antagonist

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ABSTRACT

Ciproxifan, *i.e.*, cyclopropyl-(4-(3-1*H*-imidazol-4-yl)propyloxy) phenyl) ketone, belongs to a novel chemical series of histamine H_3-receptor antagonists. *In vitro*, it behaved as a competitive antagonist at the H_3 autoreceptor controlling [^{3}H]histamine release from synaptosomes and displayed similar K_i values (0.5–1.9 nM) at the H_3 receptor controlling the electrically-induced contraction of guinea pig ileum or at the brain H_3 receptor labeled with [^{125}I]iodoproxyfan. Ciproxifan displayed at least 3-orders of magnitude lower potency at various aminergic receptors studied in functional or binding tests. *In vivo*, measurement of drug plasma levels, using a novel radioreceptor assay in mice receiving ciproxifan p.o. or i.v., led to an oral bioavailability ratio of 62%. Oral administration of ciproxifan to mice enhanced by ~100% histamine turnover rate and steady state level of *tele*-methylhistamine with an ED_{50} of 0.14 mg/kg. Ciproxifan reversed the H_3-receptor agonist induced enhancement of water consumption in rats with and ID_{50} of 0.09 ± 0.04 mg/kg, i.p. In cats, ciproxifan (0.15–2 mg/kg, p.o.) induced marked signs of neocortical electroencephalogram activation manifested by enhanced fast-rhythms density and an almost total waking state. In rats, ciproxifan enhanced attention as evaluated in the five-choice task performed using a short stimulus duration. Ciproxifan appears to be an orally bioavailable, extremely potent and selective H_3-receptor antagonist whose vigilance- and attention-promoting effects are promising for therapeutic applications in aging disorders.

HA is a cerebral neurotransmitter exerting its actions on target cells via three classes of molecularly and/or pharmacologically well defined receptors designated H_1, H_2 and H_3 (reviewed by Hill *et al.*, 1997; Schwartz *et al.*, 1991, 1995). The H_3 receptor is a presynaptic receptor regulating the synthesis and/or release of HA itself (Arrang *et al.*, 1983) as well as a variety of other aminergic or peptidergic neurotransmitters (reviewed by Schlicker *et al.*, 1994). It was initially defined by the design of two selective ligands: (R)α-MeHA, a full agonist, and thioperamide, an antagonist with nanomolar potency (K_i ≅ 4 nM). Thioperamide has become the prototypical H_3-receptor antagonist, used in a large number of neurochemical, electrophysiological and behavioral studies because it is one of the few agents able to markedly enhance cerebral histaminergic transmissions *in vivo* via a selective mechanism. In agreement, few other actions of thioperamide were described, *e.g.*, inhibition of P450 cytochromes (La Bella *et al.*, 1992) and 5-HT_3-receptor blockade (Leurs *et al.*, 1995), which require higher drug concentrations than H_3-receptor blockade and are therefore not relevant for *in vivo* studies.

Nevertheless thioperamide has several drawbacks: 1) its *in vivo* potency is rather low compared with its *in vitro* potency, suggesting that drug bioavailability, particularly its brain penetration, is restricted, 2) more importantly it displays a distinct liver toxicity on repeated administration which has prevented it being submitted to human clinical trials.

Because H_3-receptor antagonists represent a novel class of agents with potentially interesting therapeutic applications, namely in psychiatry (Schwartz *et al.*, 1995) sustained efforts have been devoted to the design of drugs more potent and safer than thioperamide (reviewed by Stark *et al.*, 1996b).

As with thioperamide, all highly effective compounds obtained so far contain a monosubstituted imidazole ring, but

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ABBREVIATIONS: HA, histamine; t-MeHA, *tele*-methylhistamine; (*R*)α-MeHA, (*R*)α-methylhistamine; AUC, area under the curve; C_{max}, maximal concentration; W, wakefulness; S1, light slow wave sleep; S2, deep slow wave sleep; PS, paradoxical sleep; EEG, electroencephalogram.

Charles River, St Aubin lès Elbeuf, France), housed in cages of 10, were allowed free access to food and water. Experiments were carried out between 10 A.M. and 1 P.M. Drugs in 0.9% NaCl solution were administered i.p., each rat receiving two injections [vehicle or (*R*)-α-MeHA 10 mg/kg/p.o., and vehicle or ciproxifan] of 250 μl each. After drug treatments, rats were returned to their home cage with food (standard pellet) and without water for 30 min. Then they were placed in individual cages with only a water supply and 10 min later, the amount of water consumed was recorded by weighing. Statistical evaluation of results was performed by Student's *t* test.

Analysis of neocortical EEG power spectral density and sleep-wake in the cat. Cats, a species in which the role of the H_3 receptor in sleep-wake control has been demonstrated, were used in this experiment (Lin *et al.*, 1990). Briefly, five adult cats of both sexes weighing 2.7 to 3.8 kg were chronically implanted, under pentobarbital anesthesia (25 mg/kg, i.v.), with electrodes for polygraphic recordings of neocortical and hippocampal EEG, ponto-geniculo-occipital activity, electromyogram and electrooculogram. In addition, a thermistor (10 K2 MCD2, Betatherm, 10 KΩ at 25°C, outer diameter of 0.45 mm) was placed in the caudate nucleus to record brain temperature. After a recovery period of 7 days the cats were housed in a sound-attenuated and dimly illuminated cage set at 24 to 26°C and fed daily at 6 P.M. with a normal standard diet. Polygraphic recordings were performed for 4 days to obtain the basic qualitative and quantitative parameters of the sleep-wake cycle.

Ciproxifan at doses of 0 (placebo), 0.15, 0.3, 0.7 and 2 mg/kg was administrated orally at 11 A.M. Subsequent polygraphic recordings were made for at least 24 hr. They were then scored minute by minute according to previously described criteria (Lin *et al.*, 1990) for wakefulness (W), light slow wave sleep (S1), deep slow wave sleep (S2) and paradoxical sleep (PS). In some animals, neocortical EEG signals from the first 6 hr after placebo or drug administration were digitized at a sample rate of 128 Hz and computed on a CED 1401 Plus (Cambridge Electronic Design, Cambridge, U.K.). The power spectral density was averaged over 30-sec epochs for the frequency range of 0.25 to 50 Hz by a Fast Fourier Transform routine using the CED program Spike2 and correlated with sleep-wake stages.

Five-choice task in rats. Male Lister hooded rats (Olac, Bicester, U.K.) were housed in pairs in a temperature-controlled (21°C) room that was illuminated in accordance with an alternating 12-hr light/dark cycle. Rats were food deprived and maintained at 85% of their free-feeding weight (MRC Diet 41B laboratory food) throughout the experiment while water was available *ad libitum*.

The test apparatus and procedure were as described in detail by Muir *et al.* (1994). Rats were trained to discriminate a brief visual stimulus presented in one of five spatial location during 30-min sessions. Rats initiated a trial by opening the magazine panel. After a 5-sec inter-trial interval a light at the rear of one of the five apertures was illuminated for 0.5 sec. Correct responses to the stimulus location were rewarded with the delivery of food pellets. Having obtained stable performance on the task (>80% correct responses), attentional demand of the task was increased by reducing the stimulus duration to 0.25 sec in certain drug sessions as described previously (Muir *et al.*, 1994, 1995). Drugs or the vehicle (saline) were administered i.p. 1 hr before the test session (0.5- or 0.25-sec stimulus duration) according to a Latin square design. A rest day followed by a baseline day separated each drug test day.

Analysis of data. Maximal effects, ED_{50}, EC_{50} and IC_{50} values were determined using an iterative computer least-squares method derived from that of Parker and Waud (1971) with the following nonlinear regression: effect of the drug = (maximal effect of the drug)·[drug dose-or-concentration]^n/([drug dose-or-concentration]^n + (ED_{50} or EC_{50} or IC_{50})^n).

K_i values of H_3-receptor antagonists are calculated from their IC_{50} values, assuming a competitive antagonism and by using the relationship (Cheng and Prusoff, 1973):

$$K_i = IC_{50}/(1+(S/K_d))$$

where S and K_d represent respectively either the concentration and the dissociation constant of the radioligand in binding experiments, or the concentration of HA and its EC_{50} in [^{3}H]HA release experiments. When a fixed concentration of ciproxifan, tested as an antagonist, was added to increasing imetit concentrations, the K_i value of ciproxifan was calculated from the following equation (Cheng and Prusoff, 1973)

$$K_i = I/[(EC_{50}'/EC_{50})-1]$$

where EC_{50} and EC_{50}' are the imetit concentrations required to obtain half-maximal inhibition of release in the absence and presence of ciproxifan respectively, and I is the ciproxifan concentration.

Radiochemicals and drugs. [^{125}I]Iodoproxyfan and [^{3}H](*R*)-α-MeHA (specific activities at reference date of 2000 and 38.0 Ci/mmol, respectively) were from Amersham (Amersham, U.K.). All drug doses are expressed as free base of compound. Administration to animals was performed with drug preparation in 1% methylcellulose for the oral route, in 0.9% NaCl for i.v. and i.p. routes. The drugs and their sources were as follows: ciproxifan [cyclopropyl-(4-(3-(1*H*-imidazol-4-yl)propyloxy)phenyl) ketone] synthesis was described in the patent application (Schwartz *et al.*, 1996); thioperamide and (*R*)-α-MeHA (Laboratoire Bioprojet, Paris, France); carboperamide [1-(heptanoyl)-4-(1*H*-imidazol-4-yl)piperidine] was from M. Robba (University of Caen, France); clobenpropit was provided by H. Timmerman (Vrije Universiteit, Amsterdam, The Netherlands); iodoproxyfan was synthesized at the Freie Universität Berlin; imetit was synthesized by S. Athmani (University College, London, U.K.). All other chemicals were obtained from commercial sources and were of the highest purity available.

Results

Effects of ciproxifan on H_3-receptor functional models in vitro. The H_3-receptor agonist imetit inhibited the [^{3}H]HA release with a maximal effect of 54 ± 2%. Pharmacological parameters were estimated using the H_3-receptor controlled inhibition curve of the [^{3}H]HA release. Imetit concentration required for half inhibitory effect of its own maximal effect was 1.6 ± 0.3 nM and the corresponding pseudo-Hill coefficient (n_H) of this concentration response curve was close to unity (0.85 ± 0.16). Ciproxifan (15 nM) induced a parallel rightward shift of the concentration-response curve for imetit and tended by itself to increase (~20%) the K^+-induced [^{3}H]HA release; the antagonism developed by ciproxifan was entirely surmounted in the presence of the highest imetit concentrations tested (fig. 2). The half-maximal inhibitory concentration for imetit in the presence of ciproxifan (estimated considering the total H_3-receptor controlled inhibition curve) was 12.8 ± 1.8 nM, leading to an apparent K_i value of 1.9 ± 0.3 nM for the antagonist.

Histamine (1 μM) inhibited its own release by 55 ± 2%, and a series of H_3-receptor antagonists progressively reversed this response with n_H coefficients close to unity (fig. 3). IC_{50} values (nM) of the various compounds were 9.2 ± 1.8 (ciproxifan), 75 ± 19 (thioperamide), 377 ± 38 (carboperamide), 11 ± 3 (clobenpropit) and 99 ± 5 (iodoproxyfan) leading to K_i values reported in table 1, assuming a competitive inhibition.

Ciproxifan (3–300 nM) competitively antagonized the (*R*)-α-MeHA induced relaxation of the electrically stimulated guinea pig ileum longitudinal muscle (Ligneau *et al.*, 1994) without significantly affecting the maximum response of the H_3-receptor agonist in the absence of ciproxifan [91 ± 5 (3 nM ciproxifan, n = 2), 100 ± 4 (10 nM, n = 6), 110 ± 10 (30 nM,

the thiourea moiety of the latter, to which hepatotoxicity might be attributable, is replaced by numerous polar functionalities such as amine or carbamate, etc.

Recently, we have designed 3-(1*H*-imidazol-4-yl)propanol derivatives as a novel series of potent *in vitro* H_3-receptor antagonists with a high capacity for oral absorption and brain penetration in some compounds (Stark *et al.*, 1996a; Hüls *et al.*, 1996). In these novel chemical classes of compounds, we have recently identified [^{125}I]iodoproxyfan, i.e., [^{125}I]3-(1*H*-imidazol-4-yl)propyl-(4-iodophenyl)methyl ether as a new probe for a sensitive assay and localization of the H_3 receptor in brain (Ligneau *et al.*, 1994).

We describe the biological properties of ciproxifan (fig. 1), a highly potent and selective H_3-receptor antagonist belonging to this novel chemical class of compounds which suggest its potential therapeutic interest as a waking and procognitive agent.

Materials and Methods

[^{3}H]Histamine release from synaptosomes. [^{3}H]HA release experiments were performed according to Garbarg *et al.* (1992). Briefly, a crude synaptosomal preparation from rat cerebral cortex was preincubated for 30 min with [^{3}H]L-histidine (0.4 μM) at 37°C. After extensive washing synaptosomes were resuspended in fresh 2 mM K^+-Krebs-Ringer's medium and in the presence of the appropriate drugs. After 5-min incubation synaptosomes were depolarized bringing the K^+-concentration to 30 mM for 2 min. Incubations were ended by a rapid centrifugation and [^{3}H]HA levels in the supernatant were determined after an ion-exchange chromatography purification. Release was expressed as the percent fraction of total [^{3}H]HA initially present in the synaptosomal preparation. Typically total [^{3}H]HA represented about 8,600 dpm/mg protein and total radioactivity about 100,000 dpm/mg protein in the test tube.

Assay of t-MeHA in brain. Male Swiss mice (18–20 g) or male Wistar rats (140–160 g) (Iffa-Credo, L'Arbresle, France) were fasted for 24 hr before p.o. administration. After treatment animals were killed by decapitation, the brain was dissected out and homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 N). The clear supernatant obtained after centrifugation (2000 × *g*, 30 min, + 4°C) was stored at −20°C before measuring the t-MeHA level by radioimmunoassay as described (Garbarg *et al.*, 1992). Changes were evaluated statistically by the Student's *t* test.

[^{125}I]Iodoproxyfan binding assays. The procedure for binding assays to rat striatal brain membranes was that described by Ligneau *et al.* (1994). Aliquots of membrane suspension (100 μl containing 15 to 20 μg of protein determined according to Lowry *et al.* (1951) using bovine serum albumin as standard) were incubated for 60 min at 25°C with 25 pM [^{125}I]iodoproxyfan (K_d = 65 ± 4 pM) alone or together with competing drugs dissolved to give a final volume of 200 μl in a phosphate buffer medium (Na_2HPO_4/KH_2PO_4 50 mM, pH 6.8). Incubations performed in triplicate were stopped by four additions of 5 ml ice-cold medium followed by rapid filtration through glass microfiber filters (GF/B, Whatman, Maidstone, U.K.) presoaked in a 0.3% polyethylene imine ice-cold buffer. Radioactivity trapped on filters was measured on a gamma counter.

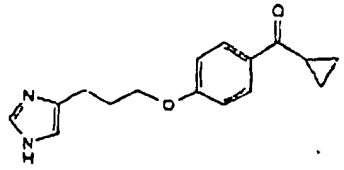

Fig. 1. Chemical structure of ciproxifan, i.e., cyclopropyl-(4-(3-(1*H*-imidazol-4-yl)propyloxy)phenyl) ketone.

Histamine H_1 receptor assay on guinea pig ileum. The procedure used was that described by Pertz and Elz (1996).

Histamine H_2 receptor assay on guinea pig right atrium. The procedure used was that described by Pertz and Elz (1996).

Muscarinic M_3 receptor assay on guinea pig ileum. The procedure used was that described by Pertz and Elz (1996).

Adrenergic α_{1D} receptor assay on rat aorta. The procedure used was that described by Hirechfeld *et al.* (1992).

Adrenergic β_1 receptor assay on guinea pig right atrium. The procedure used was that described by Pertz and Elz (1995).

Serotoninergic 5-HT$_{1B}$ receptor assay on guinea pig iliac artery. The procedure used was that described by Pertz (1993).

Serotoninergic 5-HT$_{2A}$ receptor assay on rat tail artery. The procedure used was that described by Pertz and Elz (1995).

Serotoninergic 5-HT$_3$ receptor assay on guinea pig ileum. The procedure used was that described by Elz and Keller (1995).

Serotoninergic 5-HT$_4$ receptor assay on rat esophagus. The procedure used was that described by Elz and Keller (1996).

Histamine H_3 receptor assay on guinea pig ileum. The procedure used was that described by Ligneau *et al.* (1994). Briefly, longitudinal muscle strips from guinea pig small intestine were dissected out and incubated in a gassed O_2/CO_2 (95%/5%) modified Krebs-Ringer's bicarbonate medium at +37°C in presence of 1 μM mepyramine to block the H_1 receptor. After equilibration, contractile activity under stimulation (rectangular pulses of 15 V, 0.6 msec, 0.1 Hz) was recorded. Concentration-response curves of the effect of (*R*)α-MeHA alone or together with the antagonist were established.

Radioreceptor assay of H_3-receptor ligands in serum. Male Swiss mice (18–20 g, Iffa-Credo, L'Arbresle, France) were fasted for 24 hr before ciproxifan administration. At various times thereafter, mice were decapitated. Blood was collected at +4°C, serum collected after centrifugation (1000 × *g*, 10 min, −4°C), and stored at −20°C. Ciproxifan levels were measured with the following radioreceptor assay derived from the [^{3}H](*R*)α-MeHA binding assay (Garbarg *et al.*, 1992).

Male Wistar rats (160–180 g, Iffa-Credo, L'Arbresle, France) were decapitated and the brain was removed immediately. The cerebral cortex was dissected out and homogenized in 10 volumes (w/v) of ice-cold 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5 using a Polytron. After a centrifugation (140 × *g*, 10 min, +4°C), the supernatant was recentrifuged (23,000 × *g*, 10 min, +4°C). The last pellet was washed superficially with and then resuspended in fresh ice-cold phosphate buffer to constitute the membrane fraction used for the binding assay. One-ml aliquots of the membrane suspension containing 800 to 330 μg of protein [determined according to Lowry *et al.* (1951) using bovine serum albumin as standard] were incubated for 60 min at +25°C with 1 nM of [^{3}H](*R*)α-MeHA alone or together with different concentrations of ciproxifan in diluted serum of ciproxifan-free mice (standardization curve) or with diluted serum samples of ciproxifan-treated mice. Specific binding was defined as that inhibited by 3 μM thioperamide. Incubations were performed in triplicate and stopped by four additions of 5 ml of ice-cold phosphate buffer followed by rapid filtration through glass microfiber filters (GF/C, Whatman, Maidstone, U.K.) presoaked in 0.3% polyethylene imine ice-cold phosphate buffer. Radioactivity trapped on filters was measured by liquid scintillation spectrometry (Wallac 1410, EC&G, Evry, France). The standardization curves were established using a one-site competition model (GraphPad Prism, San Diego, CA) and H_3-receptor ligand concentrations in serum were calculated using these curves and expressed as ciproxifan concentrations. In the conditions retained, the detection limit of the radioreceptor assay corresponded to a concentration of 20 nM ciproxifan in serum. Changes in H_3-receptor ligand concentrations in serum with time were analyzed using either a point to point nonlinear model or the two phase exponential decay analysis model (GraphPad Prism).

(*R*)α-MeHA-induced water consumption in rats. The procedure used was that described by Clapham and Kilpatrick (1993) with slight modifications. Briefly, male Lister hooded rats (280–320 g,

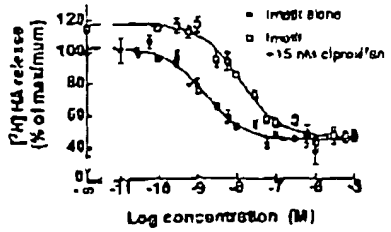

Fig. 2. Effect of ciproxifan on the inhibition by imetit of the K^+-induced [^{3}H]HA release from synaptosomes of rat cerebral cortex. Synaptosomes preincubated with [^{3}H]L-histidine were incubated for 6 min in the presence of imetit in increasing concentrations, alone or together with ciproxifan at a fixed (15 nM) concentration. They were subsequently depolarized for 2 min in the presence of 30 mM K^+ (final concentration). The spontaneous efflux of [^{3}H]HA (2 mM K^+) represented 14 ± 2% of the total [^{3}H]HA in synaptosomes. In the presence of added agents, [^{3}H]HA release induced by 30 mM K^+ (expressed in percent of total [^{3}H]HA over spontaneous efflux) represented 17 ± 2%. Each point represents the mean ± S.E.M. of results from three different experiments with quadruplicate determinations each.

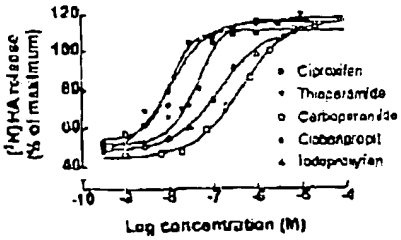

Fig. 3. Effects of H_3-receptor antagonists on the inhibition by HA of the K^+-induced [^{3}H]HA release from rat cerebral cortex synaptosomes. Synaptosomes preincubated with 1 μM HA alone or together with one of the compounds at increasing concentrations. They were depolarized for 2 min in the presence of 30 mM K^+. Each point represents the mean result from three different experiments with quadruplicate determinations each.

TABLE 1

In vitro and in vivo potencies of H_3-receptor antagonists

Compounds	*In Vitro* Tests (K_i, nM)		*In Vivo* Test (ED_{50} mg/kg, p.o.)
	[^{3}H]HA release[a]	[^{125}I]Iodoproxyfan binding[b]	Increase in t-MeHA level in mouse brain[c]
Ciproxifan	0.5 ± 0.1	0.7 ± 0.2	0.14 ± 0.09
Thioperamide	4 ± 1	4 ± 1	1.0 ± 0.5
Carboperamide	20 ± 2	6 ± 2	3.9 ± 0.8
Clobenpropit	0.6 ± 0.2	0.3 ± 0.1	~25
Iodoproxyfan	5 ± 1	0.2 ± 0.1	>25

[a] Data from Fig. 3 except for thioperamide (from Arrang *et al.*, 1987) and iodoproxyfan (from Ligneau *et al.*, 1994).
[b] Data from Ligneau *et al.* (1994) except for ciproxifan (fig. 4).
[c] Data from figure 8 except for thioperamide (from Cumellin *et al.*, 1996) and iodoproxyfan (from Stark *et al.*, 1996).

n = 6), 105 ± 9 (100 nM, n = 6) and 87 ± 7% (300 nM, n = 6) *vs.* 100% relaxation in the absence of ciproxifan). Schild analysis revealed a slope of 0.92 ± 0.04, not significantly different from unity (n = 26). After imposing the unity constraint, a pA_2 value of 8.38 ± 0.03 was calculated for ciproxifan.

Effect of ciproxifan and other H_3-receptor antagonists on [^{125}I]iodoproxyfan binding. At 25 pM [^{125}I]iodoproxyfan the specific binding to rat striatal membranes represented 8.4 ± 0.4 fmol/mg of protein, *i.e.*, 46 ± 1% of the total, and was completely and monophasically displaced by all compounds (fig. 4). From these displacement curves IC_{50} values for the compounds were deduced leading to the K_i values presented in table 1.

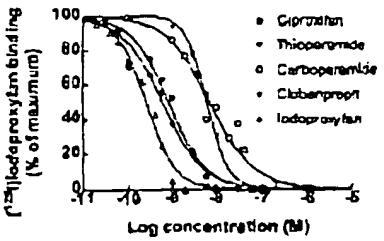

Fig. 4. Inhibition of [^{125}I]iodoproxyfan binding to rat striatal membranes by various histaminergic agents. Membranes were incubated for 60 min at 25°C with 25 pM [^{125}I]iodoproxyfan and unlabeled H_3-receptor antagonists in increasing concentrations. Specific binding, defined as that inhibited by 1 μM (R)α-MeHA, represented 46 ± 1% of the total binding. Results are expressed as percentages of specific [^{125}I]iodoproxyfan binding in the absence of unlabeled agents. Each point and vertical bars represent the mean ± S.E.M. of results from three different experiments with triplicate determinations each.

Receptor selectivity of ciproxifan. The compound displayed low apparent affinity at other receptor subtypes as evaluated in functional tests on isolated organs (histamine H_1 and H_2, muscarinic M_3, adrenergic α_{1D} and β_1, serotonin 5-HT_{1B}, 5-HT_{2A}, 5-HT_3 and 5-HT_4) (fig. 5). In addition, ciproxifan displayed a K_i value higher than 1 μM in a large variety of radioligand binding tests (Panlabs screen), except at [^{3}H]pirenzepine binding to rat cerebral cortex membranes where its K_i was about 1 μM (data not shown).

Changes in serum drug concentration in animals treated with ciproxifan. After i.v. administration of 1 mg/kg ciproxifan to mice (fig. 6B), the H_3-receptor ligand concentration in serum decreased progressively, fitting a typical biexponential decay model with half-times ($t_{1/2}$) of 13 and 87 min for the distribution and elimination phases, respectively. The quality of this fit is given by an R^2 value of 0.986. At 6 hr, the serum ligand concentration was still detectable with a value of 23 ± 6 nM. When ciproxifan was given orally, also at 1 mg/kg (fig. 5A), serum ligand level rose rapidly, being maximal at 30 min with a maximal concentration (C_{max}) value of 420 ± 40 nM; then, the ligand concentration

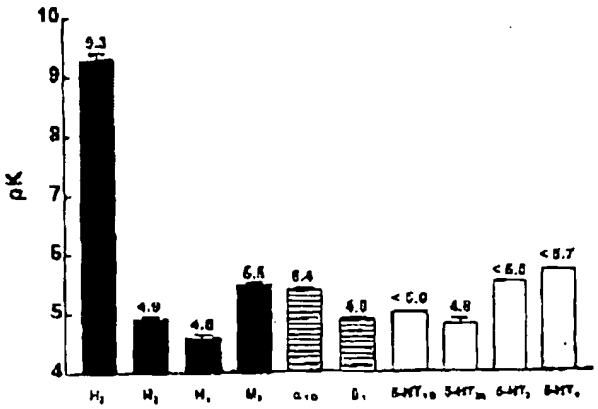

Fig. 5. Receptor selectivity profile of ciproxifan. The affinity of the compound at the H_3 receptor (pK_i from [^{3}H]HA release assay) is compared to corresponding values obtained in functional tests in isolated organs. Other values represent mean ± S.E.M. pA_2 (Arunlakshana and Schild, 1959) from n = 3 to 12 preparations except for the H_2- and β_1-receptor assay (pD'_2 according to Van Rossum, 1963).

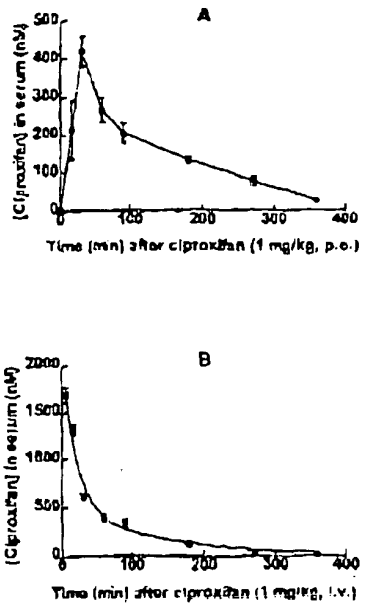

Fig. 6. Serum drug concentration in mice receiving ciproxifan. Mice were killed after p.o. (A) or i.v. (B) administration of ciproxifan (1 mg/kg), and drug concentrations were evaluated in serum by a radioreceptor assay. Means ± S.E.M. of values from six mice.

decreased but still remained measurable at 6 hr (27 ± 5 nM). The AUCs were 1425 and 690 nM.hr after i.v. and p.o. administrations, respectively, leading to an oral bioavailability coefficient (AUCp.o./AUCi.v.·100%) of 62%.

In rats (n = 3) receiving 1 mg/kg ciproxifan p.o., the time-course of changes in serum ligand concentration were comparable to those in mice with a mean AUC of 2,225 nM.h, a C_{max} of 881 ± 322 nM, also observed at 30 min, and a level of 96 ± 4 nM at 6 hr (not shown).

In one cat receiving 3 mg/kg of ciproxifan orally serum levels (μM) were of 0.14 (0.5 hr), 4.0 (1 hr), 2.2 (1.5 hr), 0.27 (3 hr) and 0.12 (6 hr) leading to an AUC of 5.07 μM.hr at this dose.

Changes in brain t-MeHA level after administration of ciproxifan or other H_3-receptor antagonists. After the administration of ciproxifan (1 mg/kg, p.o.), brain t-MeHA level rose rapidly, being already significantly increased after 30 min, reaching a plateau between 90 and 180 min and still remaining enhanced after 270 min (fig. 7). In mice receiving pargyline, a monoamine oxidase inhibitor, t-MeHA level increased linearly with time at a rate of 65 ng/g/hr, whereas coadministration of pargyline and ciproxifan enhanced this rate to 120 ng/g/hr.

The dose-response curves of ciproxifan and a series of other H_3-receptor antagonists were established by measuring t-MeHA levels 90 min after oral administration (fig. 8). Ciproxifan, thioperamide and carboperamide, maximally increased t-MeHA level to an equivalent extents about 2-fold over basal values. However, clobenpropit, at the highest dosage tested (30 mg/kg, p.o.) maximally enhanced t-MeHA level by 45%, and iodoproxyfan (10 mg/kg, p.o.) did not significantly modify this level. The ED_{50} values of the compounds, derived from data of figure 8, are reported in table 1.

Similar experiments performed in rats receiving ciproxifan orally led to ED_{50} values (mg/kg) of 0.23 ± 0.04 in cerebral cortex, 0.28 ± 0.08 in striatum and 0.30 ± 0.08 in hypothalamus with similar maximal enhancements of about 100% (not shown).

The time-course of the changes in t-MeHA levels in mouse brain elicited by oral administration of 0.3 mg/kg ciproxifan and 3 mg/kg thioperamide (fig. 9) were analyzed in terms of AUCs, leading to values (in percent increase.hr) of 597 and 425, respectively.

Effect of ciproxifan on the water consumption induced by an H_3-receptor agonist. The H_3-receptor agonist (*R*)α-MeHA (10 mg/kg, i.p.) markedly enhanced water consumption in rats, an effect that was progressively reversed by coadministration of ciproxifan in increasing dosage, the ID_{50} of the antagonist being 0.09 ± 0.04 mg/kg (fig. 10). Ciproxifan alone (3 mg/kg) did not significantly modify water consumption (fig. 10).

Effect of ciproxifan in the five-choice task in rats. Analysis of variance revealed a significant drug × stimulus duration interaction [$F(1,9) = 12.19$, $P < .01$]. As shown in figure 11, reducing the duration of the visual stimulus to 0.25 sec resulted in a significant reduction in the accuracy of performance compared to the baseline (0.5 sec) stimulus condition. Newman Keuls *post hoc* comparisons revealed that this reduction in performance was significant and that choice accuracy significantly increased after administration of 3.0 mg/kg of ciproxifan under the shorter stimulus condition compared to performance after administration of the vehicle ($P < .05$). There was no significant effect of this manipulation of the stimulus duration or of ciproxifan on any of the other measures recorded (i.e., speed, anticipatory or perseverative responses and errors of omission).

Effects of ciproxifan on neocortical EEG power spectral density and sleep-wake cycle in cat. Administration

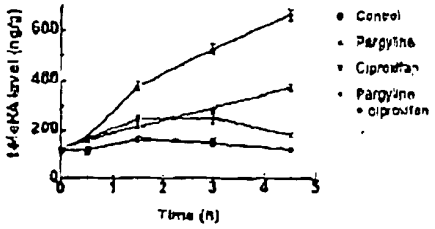

Fig. 7. Changes in brain t-MeHA levels in control and pargyline-treated mice after administration of ciproxifan. Mice were killed at various times after simultaneous administration of vehicle or ciproxifan (1 mg/kg, p.o.) and vehicle or pargyline (65 mg/kg, i.p.). t-MeHA levels are expressed in ng/g of tissue. Means ± S.E.M. of values from 12 mice.

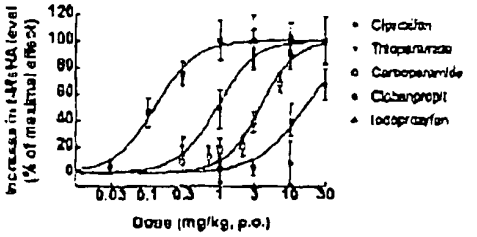

Fig. 8. Changes in brain t-MeHA levels in mice receiving H_3-receptor antagonists. Mice were killed 90 min after the p.o. administration of vehicle or drugs in increasing doses. t-MeHA levels in treated mice are expressed in percent increase as compared to levels in control mice (133 ± 7 ng/g). Means ± S.E.M. of values from 12 mice.

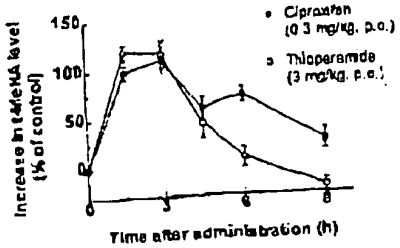

Fig. 9. Changes in brain t-MeHA levels in mice receiving ciproxifan or thioperamide. Mice were killed at various times after the p.o. administration of vehicle, ciproxifan (0.3 mg/kg) or thioperamide (3 mg/kg). t-MeHA levels are expressed in percent increase as compared to levels in corresponding controls. Means ± S.E.M. of values from 18 mice.

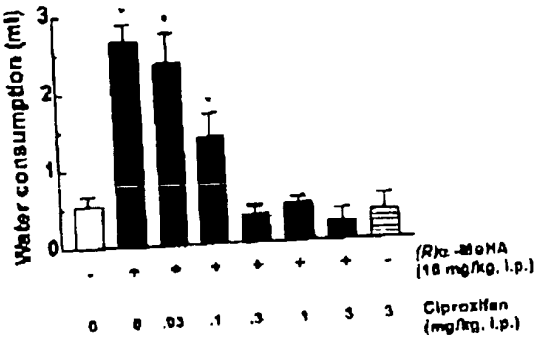

Fig. 10. Effect of ciproxifan on the (*R*)α-MeHA-induced water consumption in rats. Water consumption was measured over a 10-min period, 30 to 40 min after i.p. administrations of (*R*)α-MeHA and ciproxifan. Means ± S.E.M. of values from 5 to 10 rats per treatment group (data from two experiments). *$P < .05$ vs. vehicle.

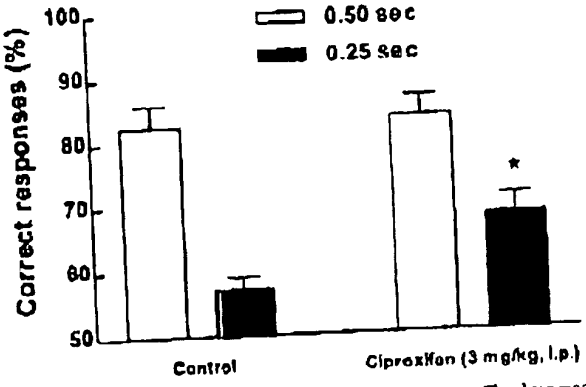

Fig. 11. Effects of ciproxifan in the five-choice task in rats. The drug was administered (3 mg/kg, i.p.) 1 hr before the test session. The accuracy of responding, expressed as the percentage of correct responses, was significantly (*$P < .05$) improved by treatment only when the stimulus duration was 0.25 sec instead of 0.50 sec.

of ciproxifan caused suppression or diminution (depending on the dose used) of neocortical slow activity (0.8–5 Hz) and spindles (8–15 Hz), resulting in a total cortical activation, *i.e.*, low voltage electrical activity with dominant waves in the β and γ bands (mainly 25–45 Hz). Furthermore, ciproxifan increased the power density of these neocortical fast rhythms (fig. 12). These effects, occurring within 25 min after

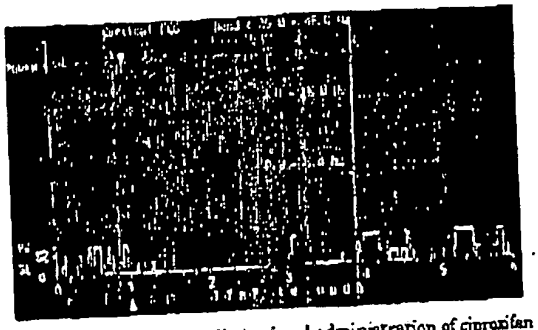

Fig. 12. An example of the effects of oral administration of ciproxifan on cortical EEG and sleep-wake cycle in cat. Neocortical EEG power density (mV^2) in different frequency bands and sleep-wake cycle 1 hr before and up to 5 hr after administration of 2 mg/kg ciproxifan (arrow) are given. Note, from bottom to top, that ciproxifan elicited a total waking state accompanied by a suppression of cortical slow frequency activity (0.8–5 Hz) and spindle (8–15 Hz) and a marked increase in fast rhythms (25–45 Hz). Abscissa, Time in hours; ordinate, sleep-wake stages (PS, paradoxical sleep; S2, deep slow wave sleep; S1, light slow wave sleep; W, wakefulness).

administration (fig. 12) were detectable at a dose of 0.15 mg/kg and become evident at a dose of 0.3 mg/kg or more (fig. 13).

The effects of ciproxifan on neocortical EEG were manifested on polygraphic scoring as an almost total waking state, the duration of which was dose dependent. This waking effect was characterized by an increase in wake-episode duration and a delayed sleep latency. During the same period both slow wave sleep (especially S2) and PS were suppressed (fig. 13). After the period of induced total waking, cortical slow activity gradually reappeared, but an increase in waking could be seen during a period proportional to the doses used. No obvious sleep rebound was noted after the waking effect, and all sleep-wake parameters including power spectral density of neocortical EEG returned to control levels on the next day. The arousal effects of ciproxifan (0.3 mg/kg) were prevented or significantly reduced by prior (15 min) systemic injection of either mepyramine (1 mg/kg), an H_1-receptor antagonist, or imetit (3 mg/kg), an H_3-receptor agonist (not shown).

Discussion

From our present *in vitro* and *in vivo* studies, ciproxifan appears as a pure competitive antagonist at the histamine H_3 receptor, one of the most potent so far available.

Our selection of the molecule was initially based on its ability to block the actions of histamine or imetit, a selective H_3-receptor agonist, at the autoreceptor regulating the release of neosynthesized [^{3}H]HA from K^+-depolarized synaptosomes, according to a previously described model (Arrang *et al.*, 1983; Garbarg *et al.*, 1989, 1992). On this model, ciproxifan induced a parallel rightward shift of the concentration-response curve to imetit, indicative of a competitive antagonism with an apparent dissociation constant in the low nanomolar range. Ciproxifan in increasing concentration also progressively blocked the (*R*)α-MeHA-induced relaxation of the electrically stimulated longitudinal muscle of guinea pig ileum in a competitive fashion. At these various functional models as well as the [^{125}I]iodoproxyfan binding tests, the drug displayed similarly low apparent dissociation constants

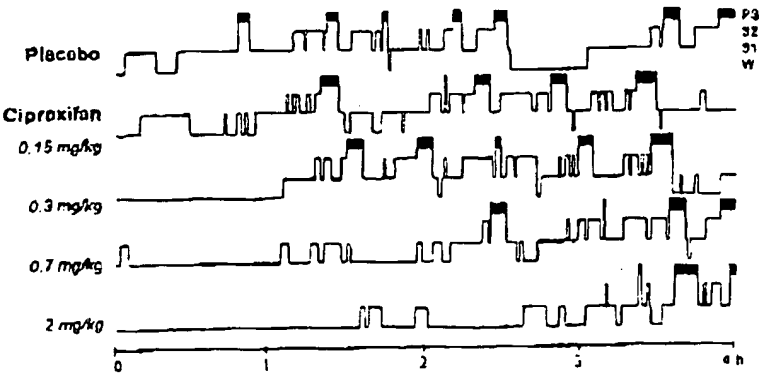

Fig. 13. Representative hypnograms (4 hr) obtained in cats after oral administration of ciproxifan at different doses. Note the dose-dependent waking effect. Abscissa, Time in hours; ordinate, sleep-wake stages (PS, paradoxical sleep; S2, deep slow wave sleep; S1, light slow wave sleep; W, wakefulness).

(K_i = 0.5–4.2 nM), an observation that does not support the hypothesis of the existence of H_3-receptor subtypes (Clapham and Kilpatrick, 1992). The hypothesis, mainly based on discrepancies in potency of some compounds in binding and functional models, is not supported either by the closely similar potencies of other antagonists in different models, exemplified in table 1 (with the exception of iodoproxyfan for which the discrepancy can be fully explained by a slow equilibration rate).

The high degree of selectivity of ciproxifan toward the histamine H_3 receptor was shown by the observation that the drug displayed lower affinity by about three orders of magnitude for any other receptor subtype on which it was tested (see "Results").

Although some compounds *in vitro* were as potent as or even more potent than ciproxifan at the H_3 receptor, *e.g.*, clobenpropit and iodoproxyfan, ciproxifan given orally to mice enhanced brain t-MeHA levels at much lower dosage (ED_{300} = 0.14 mg/kg) than any of these compounds. A similar change, occurring with ED_{nn} of 0.2–0.3 mg/kg p.o., was found in various areas of rat brain. t-MeHA is the product of the major metabolic pathway for endogenous HA in brain (Schwartz *et al.*, 1971), and its steady-state level is a reliable index of histaminergic neuron activity (Oishi *et al.*, 1983). The increase in t-MeHA level induced by the H_3-receptor antagonists corresponds to an enhanced HA release, reflecting the tonic inhibition that the endogenous amine exerts on this process and on neuronal firing via stimulation of autoreceptors in the somatodendritic or terminal area of histaminergic neurons. In agreement, ciproxifan (1 mg/kg, p.o.) enhanced HA turnover rate in mouse brain, evaluated from the rate of t-MeHA accumulation after monoamine oxidase inhibition, from a value of 55 ng/g/hr, consistent with corresponding values obtained in the same species using either isotopic (Verdière *et al.*, 1977) or nonisotopic methods (Oishi *et al.*, 1989), to a value of 120 ng/g/hr.

A similar maximal effect, corresponding to a nearly doubling of t-MeHA level, was obtained with thioperamide or carboperamide whereas, at the maximal dose tested of clobenpropit (30 mg/kg), this level was not reached and no significant change occurred after administration of iodoproxyfan (fig. 8) despite the high potency of these compounds *in vitro*.

In the case of ciproxifan, a rather high oral bioavailability was evidenced by the ratio (>60%) of AUCs of H_3 receptor binding activity in blood serum, measured by using a novel radioreceptor assay, following drug administration (1 mg/kg) by p.o. and i.v. routes, respectively. The rather slow kinetics of ciproxifan are indicated by a serum level still about 10 times above the K_i value of the drug at the H_3 receptor 6 hr after oral administration. At this time, t-MeHA levels in brain are still enhanced by 24 ± 11% (fig. 9). Such comparison between drug levels in blood and a typical brain response in rodents might be useful to predict effective dosages in other species, particularly humans, in which only blood levels are available, assuming a similar ability of the drug to cross the blood-brain barrier. A similarly favorable bioavailability of ciproxifan on oral administration to rats and cats is suggested by measurements of t-MeHA and drug serum levels, respectively (see "Results").

Characteristic behavioral responses were found in rats and cats receiving ciproxifan in low dosage. In water-deprived rats, ciproxifan blocked the enhancement of drinking elicited by (*R*)α-MeHA, an H_3-receptor agonist (Clapham and Kilpatrick, 1993), with an ID_{50} value of ~0.1 mg/kg. The exact site (central or peripheral) and mechanism of action of H_3-receptor ligands in this test has not been clearly established. Thus, whereas the involvement of the renin-angiotensin system in HA-induced drinking was postulated by Kraly and Miller (1982), an AT_1 antagonist did not affect the (*R*)α-MeHA-induced drinking (Clapham and Kilpatrick, 1993). The observation that (*R*)α-MeHA-induced drinking is blocked at doses of ciproxifan (this study), thioperamide and particularly clobenpropit (Barnes *et al.*, 1993), close to those enhancing endogenous HA release in brain (table 1), suggests that H_3 receptors in brain rather than in periphery are involved. The observation that ciproxifan or thioperamide given alone do not affect drinking suggests that the effect of (*R*)α-MeHA is mediated by H_3 hetero- rather than autoreceptors. In agreement, H_3 receptors on noradrenergic, serotoninergic, cholinergic, dopaminergic or peptidergic neurons do not appear to be, as with those on histaminergic neurons, tonically modulated by endogenous HA, because they respond to agonists but not to antagonists given alone (Schwartz *et al.*, 1991, 1995; Schlicker *et al.*, 1994).

The marked dose-dependent waking effect of ciproxifan in cats is consistent with a large variety of experimental evidence showing that histaminergic neurons play a prominent role in cortical activation and arousal in cats and rats (reviewed by Lin *et al.*, 1996; Schwartz *et al.*, 1991, 1995). The arousing effects of H_3-receptor antagonists, characterized by an enhancement of wakefulness at the expense of slow wave

and paradoxical sleep, was previously shown in both animal species using thioperamide (Lin *et al.*, 1990; Monti *et al.*, 1991) and carboperamide (Monti *et al.*, 1996). The effect of ciproxifan was, as in the case of thioperamide (Lin *et al.*, 1990), prevented by administration of mepyramine, an H_1-receptor antagonist, suggesting that it resulted from a H_3-receptor mediated enhancement of endogenous HA release. The brain site(s) at which endogenous HA promote(s) cortical EEG desynchronization via activation of the H_1 receptor could be one of the brain areas to which ascending or descending histaminergic pathways project known to express the H_1 receptor and to control sleep/wakefulness states. These potential targets comprise cortical neurons receiving direct histaminergic projections from the tuberomammillary nucleus, preoptic anterior hypothalamic neurons, thalamic relay neurons and basal forebrain or mesopontine tegmentum neurons (Lin *et al.*, 1996).

Because the effect of ciproxifan in cats was to enhance fast cortical rhythms, known to occur during increased vigilance, and to cause a quiet waking state, a positive outcome in attentional tests could be anticipated. In confirmation the drug significantly enhanced choice accuracy in the five-choice serial reaction-time task when a visual stimulus of short duration (0.25 sec) was used. Such reduction of the stimulus duration increases the attentional load placed on the task, reduces choice accuracy and has been used to observe the effects of cholinergic agents on attentional function (Muir *et al.*, 1994, 1995). "Pro-cognitive" effects of the H_3-receptor antagonist thioperamide have been reported in other behavioural tasks, *e.g.*, step-through passive avoidance response in senescence-accelerated mice (Meguro *et al.*, 1995); elevated plus-maze performance in mice with scopolamine-induced learning deficits (Miyazaki *et al.*, 1995) and in a test of social memory in rats (Prast *et al.*, 1996). However, it has also been reported that thioperamide failed to improve scopolamine-induced attentional dysfunction in the same 5-choice task used in the present study (Kirkby *et al.*, 1996).

Taken together these various observations suggest that ciproxifan is a potent, orally active H_3-receptor antagonist and it seems of interest to assess its potential therapeutic applications, namely in aging or degenerative disorders in which vigilance, attention and memory are impaired.

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Central histaminergic system and cognition

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Abstract

The neurotransmitter histamine is contained within neurons clustered in the tuberomammillary nuclei of the hypothalamus. These cells give rise to widespread projections extending through the basal forebrain to the cerebral cortex, as well as to the thalamus and pontomesencephalic tegmentum. These morphological features suggest that the histaminergic system acts as a regulatory center for whole-brain activity. Indeed, this amine is involved in the regulation of numerous physiological functions and behaviors, including learning and memory, as indicated by extensive research reviewed in this paper. Histamine effects on cognition might be explained by the modulation of the cholinergic system. However, interactions of histamine with any transmitter system, and/or a putative intrinsic procognitive role cannot be excluded. Furthermore, although experimental evidence indicates that attention-deficit hyperactivity disorder symptoms arise from impaired dopaminergic and noradrenergic transmission, recent research suggests that histamine is also involved. The possible relevance of histamine in disorders such as age-related memory deficits, Alzheimer's disease and attention-deficit hyperactivity disorder is worth of consideration, and awaits validation with clinical trials that will prove the beneficial effects of histaminergic drugs in the treatment of these diseases. © 2000 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

Although early studies suggested that histamine acts as a neurotransmitter in the brain [1], only recently has the evidence become persuasive [2,3]. In this regard, two findings have been a real breakthrough: the identification of a central histaminergic neuronal system, visualized immunocytochemically with antibodies against histidine decarboxylase [4] and histamine conjugates [5], and the discovery of the histamine H_3 receptor [6]. In the mammalian brain histamine interacts with its specific receptors, H_1 [7], H_2 [8] and H_3 [6], and with the polyamine binding site on the NMDA receptor complex [9]. This amine is involved in the regulation of numerous physiological functions and behaviors, such as thermoregulation, circadian rhythms, neuroendocrine regulation, catalepsy, locomotion, aggressiveness, drinking and feeding, learning and memory, and synaptic plasticity [2,10,11].

This review describes briefly the organization of the central histaminergic system and focuses on the involvement of this unique neuronal system in cognitive processes.

2. Histaminergic neuronal system

Cell bodies of the histaminergic neurons are located exclusively in the tuberomammillary nuclei of the hypothalamus [5,12–14]. Consistently, the mRNA for histidine decarboxylase (E.C.4.1.1.22) has been shown only in the tuberomammillary nucleus [15]. Histamine does not readily cross the blood-brain barrier, and in the brain is formed from L-histidine, which is almost exclusively decarboxylated by a specific histidine decarboxylase (E.C.4.1.1.22). Histaminergic neurons are 20–30 μm in diameter, and fire spontaneously and regularly [16]. Their efferent varicose fibers project predominantly ipsilaterally to the whole central nervous system [17–19], including most subcortical nuclei and the cerebral cortex [20]. Most of the histaminergic fibers are unmyelinated, do not surround neuronal cell bodies and make relatively few synaptic contacts, mainly with dendritic shafts [21]. Numerous synaptic contacts, though, have been observed at the electron microscope in the mesencephalic trigeminal nucleus, where dense networks of histaminergic fibers surround neuronal cell bodies [22]. Some histaminergic neurons also store other neuroactive substances and related enzymes, such as GABA [23], glutamate decarboxylase [24], adenosine deaminase [25], substance P [26] and galanin [27], in a species specific manner [28]. However, the release of these substances from histaminergic terminals has not been demonstrated yet, and the functional significance of these co-localizations is still unknown. The morphological features of the central histaminergic system, with a compact cell group and a widespread distribution of varicose fibers, resembles that of other biogenic amines, such as

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dopamine, norepinephrine and serotonin, thus suggesting that the histaminergic system may act as a regulatory center for whole-brain activity [20].

Binding studies with highly specific histamine receptor ligands in combination with light microscopic autoradiography have shown that H_1, H_2 and H_3 receptors are distributed unevenly throughout the brain. Their distribution is mostly distinct from one another and seems to be species-specific. For instance, the highest concentration of H_1 receptors in the guinea pig brain is in the cerebellum, whereas, low densities of the same receptors are found in the cerebellum of the rat. The highest H_1-receptor density in the brain of the adult rat is found in the basal hypothalamus, hippocampus, amygdala, outer layers of the cortex and pontine nuclei [29]. The primary action of the activation of H_1 receptors appears to be the breakdown of inositol phospholipids and consequent increase in cytosolic Ca^{++}. Inhibition of firing and hyperpolarization have been described in hippocampal neurons as a consequence of H_1 receptor activation [30], whereas, in other regions of the nervous system, H_1 receptor activation causes excitation through a block of potassium conductances [31]. In the guinea-pig, high levels of both H_2 receptor and its mRNA are found mostly in the striatum, limbic areas and external layers of the cortex, whereas, the septum and hypothalamus have low density of both markers [32]. Activation of H_2 receptors stimulates cAMP accumulation or adenylate cyclase activity in nervous tissue. In the central nervous system activation of H_2 receptors may block long-lasting after-hyperpolarization leading to potentiation of excitation [33], may increase the bursting activity of hippocampal pyramidal cells [34], and induce or enhance synaptic plasticity in the rat hippocampus [35].

The rat brain is rich in H_3 receptors, which appear to be widely distributed in the areas receiving histaminergic innervation [36]. Indeed, the histamine H_3 receptor was originally detected in vitro as an autoreceptor involved in the negative feedback control of histamine release at the level of histaminergic nerve-endings [6]. H_3 receptors appear to restrict the influx of calcium ions, which is essential for histamine release [6,37,38]. Thus, histamine can reduce presynaptically its own release from axonal terminals, in a fashion similar to other aminergic neurotransmitters, such as norepinephrine, through α2-receptor activation. Histamine not only inhibits its own release through the activation of presynaptic H_3 receptors, but also its own synthesis [39]. Recently, the mode of action of H_3 receptors in modulating histamine release has also been demonstrated in vivo [40–43]. Interestingly, autoradiographic studies have shown that the presence of H_3 receptors is not restricted to histaminergic neurons [44–46]. In fact, H_3 receptors function also as heteroreceptors [47], mediating histamine-induced inhibition of the release of [^{3}H]-serotonin, [^{3}H]-noradrenaline, and [^{3}H]-dopamine from slices of several rodent brain regions [48–51]. Moreover, two different laboratories have reported that H_3 receptor activation causes the inhibition of potassium-evoked release of [^{3}H]-acetylcholine from rat cortical slices [52,53]. This effect was confirmed also in vivo, using microdialysis to simultaneously administer histamine and monitor changes in acetylcholine release from the cortex of freely moving rats [54]. The inhibition of cortical acetylcholine release by H_3 receptor activation is indirect [52,54], and involves cortical GABAergic interneurons [55]. Cortical GABA interneurons control the activity of large populations of principal cells through their extensive axon arborization. Therefore, a diffuse system such as the histaminergic, may exert a powerful effect on the activity of the cortex modulating the activity of local GABA interneurons. In the rat amygdala as well histamine appears to diminish the release of acetylcholine with a mode of action which is under study [56].

3. Histamine in learning and memory

Experimental evidence indicates that central histamine may have a role in cognitive function as this amine has been shown to enhance memory (recall) in both a passive [57] and an active avoidance task [58]. H_1 receptors appeared to be involved in the memory-enhancing effect of histamine, since oral administration of H_1 receptor antagonists impaired retention of a step-through active avoidance response in rats [59]. Interestingly, both histamine and acetylcholine reversed the impairing effects of H_1 receptor antagonists [58], suggesting an interaction between central histaminergic and cholinergic neurotransmitter systems in this behavior. A memory-enhancing effect of histamine is also supported by the observation that administration of histidine, the precursor of histamine, ameliorated scopolamine-induced learning deficits in mice exposed to an elevated plus-maze test [60], and facilitated social memory in rats [61]. Consistently, depletion of histamine by the administration of α-fluoromethylhistidine, a selective inhibitor of the histamine-synthesizing enzyme [62], caused an attenuation of active avoidance acquisition [63]. However, other studies support the hypothesis that histamine impairs cognitive functions. Cacabelos and Alvarez [64] reported that administration of α-fluoromethylhistidine improved rat learning abilities in a maze paradigm, where animals had to learn to avoid a footstock. Interestingly, bilateral lesions of the tuberomammillary nucleus produced facilitation of learning [65], thus suggesting that removal of a histaminergic tone facilitates cognitive processes [10,66]. The reason for these discrepancies may be elucidated only through knowledge of the distinct and opposing modulatory actions that histaminergic tuberomammillary neurons might exert by activating different receptor subtypes on different systems involved in learning processes.

Recently, a role for H_3 receptors in cognition has been reported, and the mechanism might rest on histaminergic–cholinergic interactions [67,68]. Indeed, rat cognitive

performance in object recognition, and a passive avoidance response were impaired by pre-training administration of imetit and (*R*)-α-methylhistamine, both highly selective H_4 receptor agonists [40,69], at doses that also reduced potassium-evoked release of cortical acetylcholine [54]. Reduced availability of acetylcholine in the synaptic cleft resulted in cognitive deficits [70]. However, post-training administration of (*R*)-α-methylhistamine and imetit failed to affect rat performance in the same behavioral tasks, suggesting that H_3 receptor-induced modulation of cortical acetylcholine release influences the acquisition and not the recall processes [71]. Another histamine H_4 receptor agonist, immepip, impaired animal performance in the olfactory, social memory test, based on the recognition of a juvenile rat by a male, adult and sexually-experienced rat [61]. Conversely, (*R*)-α-methylhistamine produced a beneficial effect in rodent spatial learning and memory assessed using a water maze [72]. However, differences among the behavioral tests used may explain this discrepancy. Indeed, object recognition, passive avoidance response and the olfactory, social memory test serve to measure a form of episodic memory, possibly localized in the frontal cortex [73] and the amygdala [74], whereas, spatial learning, assessed with the water maze, is a primary function of the rodent hippocampus [75]. Lesions of the basalocortical system severely impaired object recognition, passive avoidance response and the olfactory, social memory test, but only slightly disturbed water maze performance [76], which is exquisitely sensitive to hippocampal lesions [77].

In contrast to the effects of H_3 receptor agonists, H_3 receptor antagonists appear to show beneficial effects on cognitive processes. Thioperamide, an H_3 receptor antagonist, exerted some procognitive activity in the olfactory, social memory test [61], but other studies report that the presence of any learning or memory deficit is necessary to reveal a procognitive effect of H_3 receptor antagonists. Thioperamide improved the response latency of senescence-accelerated mice (animal showing a marked age-accelerated deterioration in learning tasks) in a passive avoidance response, although it was ineffective in normally aging mice. Further, both pre-training and post-training injections of thioperamide (5 mg/kg, i.p.) or clobenpropit (15 mg/kg, i.p.) lacked any procognitive effect in control animals but, in scopolamine-treated rats (0.2 mg/kg, i.p.), both drugs completely reversed the impairments observed in object recognition and a passive avoidance response [71]. In other studies, however, administration of thioperamide (20 mg/kg, i.p.) to scopolamine-impaired mice (1 mg/kg, i.p.) attenuated only slightly scopolamine's amnestic effects in the elevated plus-maze test and the step-through passive avoidance test [67,78,79]. Differences between mice and rats may be responsible for the lower efficacy of both H_3 receptor antagonists in these studies, but it may be explained also as an effect of the use of a higher dose of scopolamine. The cognitive effects of H_3 receptor ligands might be explained by the modulation of the cholinergic system, however, they may be due to the actions of histamine on any number of transmitter systems and/or to an intrinsic procognitive role that histaminergic neurons may have as well. Indeed, a beneficial effect on a scopolamine-induced deficit is a concomitant observation, but does not prove in any way that cholinergic neurons are involved. Independently from the mechanism(s) involved, H_3 receptor antagonists may provide a novel approach to improve cognitive deficits [80,81]. This property should be particularly considered since H_3 receptors showing pharmacological characteristics and functions closely similar to those of the H_3 receptor in the rodent brain, have been identified in the human brain [82].

4. Histamine and attention-deficit hyperactivity disorder

Attention-deficit hyperactivity disorder (ADHD) is characterized by psychomotor agitation, impulsive behavior, hyperactivity, inattention, learning disorders and disorganized behavior. The age of onset is early childhood, but symptoms continue to persist in adolescence, often resulting in academic, social and emotional problems [83]. The outcome in adulthood shows that 30–70% of children with ADHD will experience persistent symptoms of the condition as adults [84]. A genetic predisposition has been implicated in the etiology of ADHD, but environmental factors, such as socioeconomic status, composition, structure and emotional aspects of family, appear to influence the outcome [85]. Interestingly, many ADHD symptoms resemble prefrontal cortex dysfunction. Indeed, humans [86–88] and animals [89] with prefrontal cortical lesions, especially those involving the right hemisphere [90], show deficits, such as poor attention regulation, disorganized behavior, hyperactivity and impulsivity, which parallel those observed in ADHD patients. More precisely, lesions of the right orbital prefrontal cortex cause lack of restraint and psychomotor agitation [91], and lesions of the right dorsal prefrontal cortex impair attention [92] and inhibition to distracting stimuli [93]. Moreover, a report that ADHD patients are consistently impaired on tests of frontal lobe function [94] but perform normally on tests of parietal attentional abilities [95], confirms the implication of prefrontal cortical mechanisms. Interestingly, the right prefrontal cortex is smaller [96], and the cerebral activity in striatal and posterior periventricular regions was lower [97] in ADHD patients than in age-matched controls.

Experimental evidence suggests that ADHD symptoms arise from impaired dopaminergic [98–100] and noradrenergic [98,101] transmission, and ADHD is generally treated with psychostimulants, such as methylphenidate, that promotes catecholaminergic transmission. The positive effects might be due to normalization of an asymmetry in the prefrontal cortex and caudate [102–104]. However, the exact mechanism by which psychostimulants act as calming agents in ADHD patients is still unknown, and a role of

serotonin has also been claimed [105]. Recently, a new model for understanding ADHD has been proposed which implies a hypothalamic dysfunction resulting in disturbance of the arousal level [106].

As mentioned before, evidence from several laboratories indicates that the histaminergic system is involved in modulation of attention and vigilance, of cognition and of release of neurotransmitters, such as acetylcholine, dopamine, noradrenaline and serotonin [3,107–109]. These findings may be relevant to our understanding of ADHD. In this regard the studies of Tedford and his colleagues at Gliatech are very interesting. Based on the biochemical time course development of the monoamine systems [110], this group has established a juvenile rat pup model possibly related to ADHD [81]. Cognitive impairment have been reported in developing rat pups [111], and attention deficit disorders with hyperactivity have been produced in various juvenile rat models [100,112,113]. Juvenile rat pups' rate of acquisition of a multi-trial step-through passive avoidance response was improved significantly by pre-training administration of GT2016 [81], a selective H_3 receptor antagonist, at doses that also paralleled cortical H_3 receptor occupancy profiles and enhanced cortical histamine release in vivo [114]. Methylphenidate, a psychostimulant used for the treatment of ADHD, improved acquisition in the learning impaired juvenile rat pups similarly to GT2016 [81]. These encouraging results in animal models await validation with clinical trials that will prove the beneficial effects of H_3 receptor antagonists in the treatment of ADHD.

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Interactions between histaminergic and cholinergic systems in learning and memory

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Abstract

The aim of this review is to survey biochemical, electrophysiological and behavioral evidence of the interactions between the cholinergic and histaminergic systems and evaluate their possible involvement in cognitive processes. The cholinergic system has long been implicated in cognition, and there is a plethora of data showing that cholinergic deficits parallel cognitive impairments in animal models and those accompanying neurodegenerative diseases or normal aging in humans. Several other neurotransmitters, though, are clearly implicated in cognitive processes and interact with the cholinergic system. The neuromodulatory effect that histamine exerts on acetylcholine release is complex and multifarious. There is clear evidence indicating that histamine controls the release of central acetylcholine (ACh) locally in the cortex and amygdala, and activating cholinergic neurones in the nucleus basalis magnocellularis (NBM) and the medial septal area-diagonal band that project to the cortex and to the hippocampus, respectively. Extensive experimental evidence supports the involvement of histamine in learning and memory and the procognitive effects of H_3 receptor antagonists. However, any attempt to strictly correlate cholinergic /histaminergic interactions with behavioral outcomes without taking into account the contribution of other neurotransmitter systems is illegitimate. Our understanding of the role of histamine in learning and memory is still at its dawn, but progresses are being made to the point of suggesting potential treatment strategies that may produce beneficial effects on neurodegenerative disorders associated with impaired cholinergic function. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Acetylcholine; Histamine; Amygdala; Hippocampus; Cerebral cortex; Release

1. Introduction

The extensive loss of cholinergic neurons in the basal forebrain, detected at autopsy [104] and, more recently, using chemical imaging [73], is the most salient neurochemical feature of Alzheimer's disease [27], and has been linked to cognitive impairment [105]. Furthermore, both cholinergic [32] and memory deficits [72] occur also in normal aging, although these dysfunctions differ qualitatively and quantitatively from those reported in AD. These observations, together with a wealth of data showing that anticholinergic drugs, such as scopolamine and atropine, produced learning and memory deficits [25,35,44], have led to the cholinergic hypothesis of geriatric cognitive dysfunction [10]. As a result, much of the research on cognitive decline has focused on the role of central acetylcholine (ACh) [41], and related treatment strategies have traditionally aimed at restoring the cholinergic neurotransmission. However, therapies with cholinesterase inhibitors or muscarinic agonists have been generally unproductive [69], being improvements of cognitive functions generally modest and confined to a minority of patients, although whether such therapies provide protection against further cognitive decline is still being evaluated [103]. These drugs may disrupt the normal pattern of cholinergic transmission, thus blocking proper signal

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processing. In addition, over the past decade much evidence has shown that the 'cholinergic hypothesis of learning' [10] is too reductionistic. [58,112]. Different pathological manifestations of AD, such as β-amyloidosis, presence of tangles and dystrophic neurites, synapse loss and various neurotransmitters deficits render unlikely that cholinergic dysfunction could account for all cognitive and non-cognitive symptoms. Furthermore, several neurotransmitters, including dopamine, GABA, noradrenaline, serotonin and histamine, are clearly implicated in cognitive processes and interact with the cholinergic system [33,99]. Since abnormalities of these neurotransmitter systems have been identified in Alzheimer's disease and aging [1,57], these alterations might well interact with those of ACh to cause additive or even synergistic effects on cognition. For instance, the role of serotonin in learning and memory has received much interest [76], although the data appear to be rather inconsistent [76]. Nevertheless, $5HT_3$ antagonists seemed to improve the performance of rodents and primate in various cognitive tests [102], possibly through modulation of cholinergic mechanisms [52]. Also dopamine, especially the mesocortical system, is thought to have a crucial role in learning and memory [74]. Increased dopamine turnover in the prefontal cortex impaired spatial memory performance [88]. In the auditory cortex an increase of dopaminergic activity evaluated by microdialysis appeared to reflect the initial formation of the behaviorally relevant association [120], and the phasic activation of mesocortical and mesolimbic dopaminergic systems is differentially influenced by associative and non associative learning mechanisms [11]. Histamine implications in learning and memory is supported by extensive experimental evidence [94,124], and there is evidence that histaminergic H_3 receptor antagonists facilitated memory acquisition [51], possibly through cholinergic mechanisms [99]. Interestingly the effect of tacrine (1,2,3,4-tetrahydroamino-9-acridinamine), which alleviates Alzheimer's disease symptoms in some patients, may be partly due to multiple pharmacological mechanisms. Although protection of endogenous ACh is its most accepted mechanism of action, through acetylcholinesterase inhibition, tacrine was more potent to inhibit histamine-*N*-methyltransferase, the enzyme responsible for brain histamine metabolism, than acetylcholinesterase [86]. The enhancement of histamine brain level might be related with the activity of tacrine in Alzheimer's disease. Thus, the role of interactions between ACh and other neurotransmitters affecting cognition is of considerable interest. This review focuses on interactions between the cholinergic and the histaminergic systems and examines the possible role of such interactions in learning and memory.

2. Modulation of hippocampal cholinergic tone by histamine

The cholinergic system might be one of the most important modulatory neurotransmitter systems in the brain. It is distributed in a variety of different nuclei, two groups of which are localized in the forebrain. The nucleus basalis magnocellularis (NBM) is the major source of cholinergic innervation to the neocortex, and the amygdala, whereas the medium septum-banda diagonalis complex (MSA-DB) provides cholinergic input to the hippocampus [78,89,135]. Since degeneration of these two cholinergic pathways is the most consistent damage occurring in Alzheimer's disease [27], a large number of studies have investigated the regulation of either NBM or MSA-DB cholinergic neurons. These neurons appear to be interconnected with several neurotransmitters, such as dopamine, noradrenaline, serotonin, GABA, opioids, galanin, substance P and angiotensin II [33]. There is also much evidence suggesting that histaminergic system modulates both NBM and MSA-DB cholinergic pathways [13,100]. Indeed, histaminergic cell bodies are exclusively localized in the tuberomammillary nucleus of the hypothalamus [97,131], from where they project efferent fibers, pre-

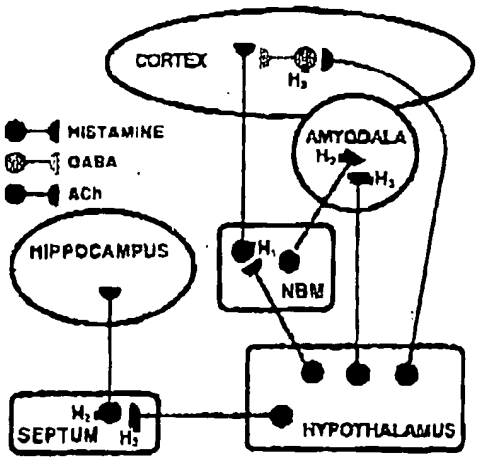

Fig. 1. Schematic diagram of the sites at which cholinergic/histaminergic interactions might occur. Three systems may be of special interest, the nucleus basalis magnocellularis (NBM)/cortical system, the NBM/amygdaloid system and the medium septum-banda diagonalis (MSA-DB)/hippocampal system. Drugs acting at H_1 receptors may affect cortical cholinergic tone by interacting with cholinergic perikarya in the NBM, whether directly or indirectly is not known yet. H_3 receptor agonists or antagonists may affect the cholinergic tone in the amygdala at cholinergic terminals level. They may also modulate the hippocampal cholinergic tone by interacting with cholinergic perikarya in the MSA-DB, whether directly or indirectly is not known yet which. H_3 receptor ligands may interact with autoreceptors in the septum and the amygdala, thus modulating indirectly the cholinergic tone in the amygdala and the hippocampus. They may also thus affect cortical cholinergic activity by acting at postsynaptic H_3 receptors localized on GABAergic neurons.

dominantly ipsilaterally and with multifold arborizations, into the whole central nervous system, including the NBM, MSA-DB, amygdala, hippocampus and cerebral cortex [64,96,123,130]. Fig. 1 shows a schematic diagram of the sites at which cholinergic/histaminergic interactions might occur.

Hippocampus has long been thought to be an important cortical region for associative learning and memory. An early study indicated that ACh release from the CA1–CA3 region of hippocampus of anesthetized rats could be modulated by endogenous histamine [83]. Indeed, an electrical stimulation applied to the tuberomammillary nucleus greatly increased both histamine release from MSA-DB and ACh release from hippocampus. Similar results were obtained when the hypothalamus was perfused with a 100 mM potassium-containing medium [83]. ACh release was increased through the release of histamine, since both ACh and histamine electrically-evoked releases were abolished in rats pretreated with α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase. This enzyme is essential for histamine synthesis [71], and its blockade caused a complete depletion of neuronal histamine [132]. ACh electrically-elicited release was inhibited by systemic administration of zolantidine, an H_3 receptor antagonist [21], but not of pyrilamine, an H_1 receptor antagonist, thus indicating that activation of H_3 receptors resulted in an increase of extracellular level of hippocampal ACh [83]. Stimulation of H_3 receptors released also endogenous noradrenaline from rat hypothalamic slices [16] and prolactin [34]. The implication of endogenous histamine was further supported by the observation that administration of thioperamide, an H_3 receptor antagonist [4], increased, while that of R-α-methylhistamine, an H_3 receptor agonist [4], decreased ACh spontaneous release from hippocampus [83]. Indeed, the H_3 receptor was initially discovered on histaminergic neurons as a presynaptic autoreceptor, whose activation inhibited the release of histamine, and its blockade elicited an increase of histamine extracellular levels [4,5].

Electrical stimulation of tuberomammillary nucleus enhanced histamine release not only from MSA-DB, but also from hippocampus, thus indicating that it might act at both the cell bodies and the terminals of the cholinergic system [83]. However, since the electrical stimulation elicited a response in histamine release from MSA-DB six-fold greater than that elicited from hippocampus, it is perhaps more likely that an interaction between histaminergic and cholinergic systems occurred in the MSA-DB complex. These observations have been largely confirmed and extended, and it is clear yet that histamine exerts a tonic influence on hippocampal cholinergic activity only at MSA-DB-complex level. In fact, microdialysis experiments have failed to show an effect of histamine, applied locally to hippocampus, on extracellular level of hippocampal ACh [8]. In the extension of this study, histamine receptor-selective compounds were applied by retrograde microdialysis to the MSA-DB area of the rat brain, and the effects of this infusion on extracellular ACh in hippocampus were recorded with a second microdialysis probe [7]. Intraseptal administration of thioperamide increased significantly the spontaneous release of ACh from hippocampus of freely moving rats by up to about 100% [7]. Cimetidine, an H_2 receptor antagonist [38], fully antagonized the effect of thioperamide. Also cimetidine was administered locally into the septum [7]. Thus, assuming that intraseptal administration of thioperamide produced an increase of endogenous histamine extracellular levels, this study further supports the suggestion that histaminergic neurons projecting to the septum [96] facilitate hippocampal cholinergic activity. The blockade of thioperamide effect on hippocampal ACh release by cimetidine suggests that endogenous histamine interacted with postsynaptic H_2 receptors, although it is not yet clear whether H_2 receptors are located on the septal cholinergic cell bodies, or on hypothetical neurons, which in turn facilitate the release of hippocampal ACh. Also intraseptal administration of ciproxifan, another H_3 receptor antagonist [75], increased ACh spontaneous release from hippocampus of freely moving rats by about 100%, and its effect was fully antagonized by cimetidine [6], thus confirming the hypothesis that histaminergic efferents to the septum facilitate hippocampal cholinergic activity through H_2 receptor activation.

Electrophysiological findings indicated that histamine depolarized MSA-DB cholinergic neurons in a slice preparation of rat brain, producing an increase in sodium conductance which led these neurons to threshold for firing spontaneous action potentials [54]. The effect was attributed to H_1 receptor activation, since it was significantly reduced by mepyramine (also known as pyrilamine) and promethazine [54], both H_1 receptor antagonists [61]. However, the concentrations employed were very high, and the authors may have underestimated additional nonspecific properties of these two compounds [60]. Moreover, depolarization induced by histamine was transient, being desensitization within seconds, its prominent feature and the excitation diminished despite continued application of histamine [54]. If the effect on ACh release was very rapid, it is possible that it became obscured during the attainment of the 20-min perfusion sample, thus explaining why the biochemical studies mentioned above have failed to find an effect of H_1 receptor blockade on septohippocampal cholinergic activity. In contrast to earlier cited microdialysis studies, Dringenberg and colleagues [36] demonstrated that systemic administration of mepyramine caused a very large increase of ACh spontaneous release from hippocampus of urethane-

anesthetized rats, thus suggesting an implication also for H_1 receptors. The discrepancy between this study and Mochizuki's work [83], which failed to show any effect of mepyramine, may be related to differences in the doses used, 10–20 and 5 mg/kg, respectively. Moreover, these H_1 receptor antagonists possess marked antimuscarinic properties [59,60], and consequently their selectivity between the three different histamine receptors [61] does not guarantee an unambiguous characterization.

3. Modulation of *c-fos* expression by histamine

In addition to the effect on hippocampal ACh release, *c-fos* immunoreactivity was detected in the medial septum 90 min after intraseptal administration of ciproxifan [6]. Significantly fewer *c-fos* immunoreactive nuclei were seen in control. Also the effect of ciproxifan on *c-fos* expression was fully antagonized by cimetidine [6]. Morphological features indicate that *c-fos* was expressed in neuronal cells, but the type of neuron has not been identified yet. The protooncogene *c-fos* is an immediate-early gene linked to genomic events in the cellular response to environmental signals [116], and has provided a useful marker for tracing the effects of pharmacological, electrical and physiological stimuli in the CNS [85]. Although increased hippocampal ACh release and *c-fos* expression might be dissociated processes, despite the identity of the stimulus, these observations may have implications for the treatment of disorders associated with impaired septo-hippocampal cholinergic functions.

4. Modulation of cholinergic tone in the cortex and in the amygdala by histamine

In addition to the findings indicating interactions between histamine and the septo-hippocampal cholinergic pathway, there is also evidence that histamine may have a regulatory role on the release of ACh also in the NBM-cortical and the NBM-amygdaloid pathways. Two different laboratories reported that histamine inhibited potassium-evoked release of [^{3}H]-ACh from rat cortical slices preloaded with [^{3}H]-choline through activation of H_3 receptors [3,23]. The effects of histamine and agents acting at histamine receptors on spontaneous and potassium-evoked release of ACh were also investigated in vivo, using microdialysis to simultaneously administer histamine and monitor changes in endogenous ACh release from cortex of freely moving rats [14,15]. Histamine, administered locally into the cortex, failed to affect ACh spontaneous release. Conversely, it inhibited concentration-dependently potassium-elicited release of ACh. The H_3 receptor agonists R-α-methylhistamine, imetit [46,62,126], and immepip [128] mimicked the effect of histamine, showing a slightly greater potency than histamine [14]. Oppositely, neither 2-thiazolylethylamine [45], an agonist showing some selectivity for H_1 receptors, nor the H_2 receptor agonist dimaprit [98] modified potassium-evoked release of ACh [14]. The inhibitory effect of 100 μM histamine, a concentration producing the maximal effect [14], was completely prevented by histamine H_3 receptor antagonists, such as clobenpropit [126] and thioperamide, but was resistant to antagonism by triprolidine [65] and cimetidine, antagonists at histamine H_1 and H_2 but not H_3 receptors [14,15]. All agonists and antagonists were administered locally, dissolved into the perfusion medium. The concentration of potassium used in these studies, 100 mM, is only apparently high, for the low recovery of potassium through the microdialysis membrane [134], and the rapid dilution of potassium in the extracellular space necessitate high concentrations in the perfusion fluid. In fact, 60 mM potassium had only a slight effect on ACh release during brain dialysis [133], and perfusion of the cortex in vivo with 100 mM potassium evoked an increase in ACh release [14,15] similar to that obtained with incubation of cortical slices in 20 mM potassium [23]. H_3 receptor-induced inhibition of potassium-evoked release of ACh was completely abolished in cortices in which the traffic of action potentials was blocked by tetrodotoxin, a voltage-dependent sodium-channel blocker [14]. Thus, H_3 receptors modulating ACh release are likely located neither presynaptically on cholinergic nerve terminals, nor on non-cholinergic nerve endings impinging on the former. They are most likely somatodendritic receptors on interneurons, the excitation of which produced sodium-dependent action potentials that release an intermediary modulatory substance. Consistently, in synaptosomes of entorhinal cortex, the release of [^{3}H]-ACh remained unaltered in the presence of two H_3 receptor agonists [3], thus strongly suggesting that H_3 receptors modulating cortical ACh release are located postsynaptically on intrinsic perikarya [3]. Indeed, H_3 receptors are not restricted to extrinsic histaminergic nerve endings [107], and H_3 receptor-mediated inhibition of the release of neurotransmitters other than histamine has been described [113]. Moreover, lesion experiments demonstrated that H_3 heteroreceptor number present on intrinsic neurons or other target cells is, at least in some regions, much greater than that of H_3 autoreceptors [107]. Consistently, degeneration of perikarya by local infusion of kainate strongly decreased the number of H_3 receptors in the striatum and the cerebral cortex [26,107].

Recent microdialysis experiments demonstrated that bicuculline, a $GABA_A$ receptor antagonist, reversed the inhibition of ACh release induced by immepip, an H_3 receptor agonist, thus suggesting a GABAergic involve-

ment [48]. Furthermore, immepip, at a concentration that produced a maximal inhibition of potassium-evoked ACh release [14] increased 100 mM potassium-evoked release of GABA from the cortex of freely moving rats by more than 50% [48]. Thus it is conceivable that H_3 receptors, localized postsynaptically on intrinsic perikarya, facilitated GABA release, which, in turn, inhibited ACh release. The most simple hypothesis is that GABA activated $GABA_A$ receptors localized on cholinergic nerve endings, thus reducing ACh release. Experimental evidence suggests that the cortical GABAergic system exerts a tonic inhibition of spontaneous release of ACh from the cortex, and that this inhibitory tone is maximal [49]. This could elucidate why neither histamine nor either of H_3 receptor agonists altered spontaneous ACh release [14], much of which is tetrodotoxin sensitive [14]. Under resting conditions, since the inhibition of ACh release caused by GABA is maximal, H_3 activation would have no effect on spontaneous ACh release. However, activation of H_3 receptors, by increasing the release of GABA, will antagonize the potassium-induced depolarization, thus, depress, at least partially, potassium-evoked acetylcholine release. Alternatively, another synaptic arrangement consonant with the lack of H_3 modulation of spontaneous release is that the activated interneurons inhibit the release of an excitatory presynaptic modulator of cholinergic terminals. If this excitatory pathway were not spontaneously active, H_3 activation would have no effect on spontaneous ACh release. In the presence of potassium, this excitatory modulator would be released and enhance the depolarization-induced release of ACh. Activation of H_3 receptors would remove this enhancement and partially, but not completely, depress potassium-evoked ACh release. Cortical GABA interneurons control the activity of large populations of principal cells through their extensive axon arborization [42]. Therefore, any pathway, even if relatively sparse such as the histaminergic pathway, may exert a powerful effect on the activity of the cortex if it modulates the activity of local GABA interneurons.

Histaminergic modulation of cortical cholinergic tone appears to be complex and multifaceted, and consists of two components, one inhibitory related to local actions at the terminals, the other excitatory resulting from interactions with cholinergic cell bodies in the NBM. Indeed, an electrophysiological study in guinea-pig basal forebrain slices, reporting that histamine depolarized NBM cholinergic mainly through H_1 receptor activation [70], suggests that histaminergic neurons might also facilitate cortical cholinergic release. An intact, whole animal approach yielded important insight into the physiological role of histamine in modulating cortical cholinergic activity, rats were implanted with two microdyalisis probes; one in the NBM to deliver locally the different drugs; and the other in the cortex to measure the output of ACh [22]. The administration of histamine into the NBM increased concentration-dependent the output of ACh from the cortex of freely moving rats by about 100% [22]. ACh release elicited by 100 μM histamine was insensitive to blockade of H_2 and H_3 receptors by means of cimetidine and thioperamide [22]. Conversely, triprolidine, an H_1 receptor antagonist, reduced significantly the effect of 100 μM histamine [22]. Although, mechanisms concomitant to H_1 receptor activation cannot be excluded, both electrophysiological and biochemical findings indicate that histamine in the NBM facilitates the cortical cholinergic activity, and strongly suggest that H_1 receptor activation is, at least in part, responsible for this effect. The dual effect of histamine on cortical cholinergic activity, excitatory at the level of NBM cell bodies, and inhibitory at the level of cholinergic terminals [14,48], may have implications for the treatment of disorders associated with impaired cortical cholinergic functions.

The amygdala is involved in the cognitive evaluation of the emotional content of complex cues, and acquisition of characteristic responses to aversive events depends on its integrity [122]. The basolateral nuclei, which receive major inputs from cortical and subcortical sensory areas [29], also receive cholinergic innervation from NBM [78] and histaminergic innervation from the hypothalamus [96]. Furthermore, autoradiographic and immunohistochemical studies have shown high densities of both H_3 [107] and muscarinic receptors in this brain region [127]. Modulation of cholinergic transmission in the amygdala may be important for the acquisition or expression of relevant behaviors. Local administration of thioperamide decreased significantly the spontaneous release of ACh from basolateral nuclei of freely moving rats by about 50% [101]. This effect was fully blocked by cimetidine [101]. The inhibitory effect of thioperamide on ACh release may be explained by an interaction with H_3 autoreceptors. Blockade of these receptors caused an increase of extracellular levels of endogenous histamine [5]. Therefore, this study suggests that activation of histaminergic neurons projecting to the amygdala basolateral nuclei inhibits the cholinergic tone in this area. Postsynaptic H_2 receptors seem to mediate this effect, since pretreatment with cimetidine fully antagonized the effect of thioperamide. Whether H_2 receptors are located on cholinergic terminals, or on hypothetical interneurons is not clear yet.

5. Histamine and cognition

Despite the complexity of the neuromodulatory relationship between cholinergic and histaminergic systems, a clear connection between histamine and learning and memory-related processes is provided by its involve-

ment in the induction of long term potentiation (LTP) in area CA1 of rat hippocampal slices [19]. LTP has been suggested to be the physiological correlate of memory formation [17]. Histamine-induced modulation of synaptic plasticity is not consequent to activation of classical histamine H_1, H_2 or H_3 receptors [19], but it is attributable to an interaction with the polyamine-binding sites on the NMDA receptor complex [12,129]. However, behavioral studies on animal models have provided extensive experimental evidence that the classical histamine receptors are also involved in histamine effects on learning and memory. For example, immediate post-training administration of histamine facilitated retention of a step-down inhibitory avoidance behavior, and this effect was antagonized by the simultaneous administration of both prometazine, an H_1 receptor antagonist, and cimetidine, an H_2 receptor antagonist [30,31]. Consistently, histamine improved the response latency in a one-way active avoidance response of aged rats [124]. This effect was mimicked by H_1 receptor agonists, and antagonized by H_1 receptor antagonists [124]. Aged animals are impaired in the acquisition of several learning procedures, such as both active and passive avoidance or maze learning [124]. Central histamine receptors are implicated also by findings that oral administration of classical H_1 receptor antagonists, such as mepyramine and promethazine retarded the acquisition and impaired the retention of acquired learning in an active avoidance task, while H_1 receptor antagonists less liable to cross the blood brain barrier, such as astemizole and oxatomide, caused only a weak depression of the avoidance response [66]. Interestingly, histamine as well as acetylcholine antagonized the effects of mepyramine [68], thus indicating that histaminergic and cholinergic central systems might exert a functional interaction in this behavior. In fact, although mepyramine's antimuscarinic properties are well known, it seems unlikely that they accounted for this effect, since the dose of acetylcholine required for preventing mepyramine-elicited amnesia was clearly very high, ten times higher than that able to antagonize the inhibitory effect of atropine [68]. In these studies histamine was always administered intracerebroventricularly, since this amine doesn't cross the blood–brain barrier [55]. It is, however, dubious whether or not injection of histamine truly reflects the actions of endogenous histamine in the brain. A possible answer to this question arises from investigations on the effects of L-histidine. In fact, histamine in brain is formed from L-histidine, which is taken up by an active process, and decarboxylated by a specific L-histidine decarboxylase (EC 4.1.1.22), which is not saturated under normal conditions [56]. Therefore, administration of L-histidine raised brain histamine levels [115]. Administration of L-histidine to hippocampus-lesioned rats amply increased hippocampal histamine content, and reduced significantly the lesion-induced deficits of both acquisition and retention of an active avoidance response [124]. L-Histidine also ameliorated learning deficits induced by scopolamine in mice exposed to an elevated plus-maze test [79], and was effective in improving rat learning performances in the olfactory social memory test [108], which is based on the investigation time of a juvenile rat by an adult rat, and measures a form of short-term memory [125]. These improvements of learning behaviors were mediated by newly synthesized brain histamine, since they were prevented by pretreatment with α-fluoromethylhistidine [79,124], although peripherally administered histamine was ineffective [79]. Mepyramine antagonized L-histidine ameliorating effects, thus confirming a role of central H_1 receptors [79]. The blockade of L-histidine decarboxylase by α-fluoromethylhistidine lowered histamine content in those cells, such as histaminergic neurons, where the amine turned over rapidly [132]. Administration of α-fluoromethylhistidine produced a significant suppression of memory retrieval and learning acquisition of active avoidance response [67,124]. Interestingly, the duration of the response latency was highly correlated with the depletion of histamine content in specific brain areas, such as hippocampus and hypothalamus [67,124]. It is important to remark that effective doses of α-fluoromethylhistidine failed to influence locomotor activity [67,93], thus supporting the hypothesis that the decrease of neuronal histamine was directly responsible for cognitive impairments. In contrast to the hypothesis that histamine improves cognitive function, other studies implicate a negative role of histamine on learning and memory processes. Rats treated with α-fluoromethylhistidine showed increased learning abilities in a maze paradigm, where they had to learn to avoid a foot shock [20]. Moreover, localized histamine injections into rat hippocampus prolonged significantly the latency time to escape in an active avoidance response [2]. Recently, the effects of lesions of the tuberomammillary nucleus on the performance of adult and aged rats in a set of cognitive tasks have been reported; in addition to a marked decrease in the number of histaminergic neurons, these lesions produced an improvement in every cognition test applied, and strongly diminished the age-related learning deficits [43]. Since amplification of the reward after hypothalamic stimulation was demonstrated following bilateral lesions of the tuberomammillary nucleus [63], one might suggest that tuberomammillary nucleus lesions facilitated cognition by enhancing the function of the reinforcement system. Interestingly, the implication of these studies, that the histaminergic system might exert an inhibitory tone on cognitive processes, could be readily integrated with findings that brain histaminergic activity was higher in the elderly [109,110], and histamine content of rat brain increased with age [92]. In conclusion, a role of his-

Table 1
Effects of H_3 receptor antagonists on cognitive tasks

Drug	Behavior test	Effect	Reference
Ciproxifan	Five-choice	Improvement	[75]
Clobenpropit	Passive avoidance	Improvement	[51]
	Object recognition	Improvement	[51]
FUB 181	Elevated plus-maze	Improvement	[91]
Thioperamide	Social memory test	Improvement	[108]
	Passive avoidance	Improvement	[51,77]
	Object recognition	Improvement	[51]

tamine in learning and memory processes is highly probable, but at present experimental evidence appears to be inadequate to enable firm conclusions to be drawn on this role. The knowledge of the distinct and possibly opposing modulatory actions that histaminergic tuberomammillary neurons might exert by activating different receptor subtypes on specific neuronal networks involved in different learning processes may help resolve the controversy concerning its role in cognition.

Promising data have been obtained with H_3 receptor antagonists, which have been found to improve the performance of rodents in various cognitive tests (Table 1). Indeed, thioperamide, an H_3 receptor antagonist, improved rat performance in the olfactory, social memory test [108]. In rats, ciproxifan enhanced attention as evaluated in the five-choice task performed using a short stimulus duration [75]. Ciproxifan is a potent and selective H_3 receptor antagonist, which, being orally bioavailable, appears promising for therapeutic applications in aging disorders. Other studies, however, reported that the procognitive effects of H_3 receptor antagonists became fully evident only when behavioral deficits were pronounced. For example, while thioperamide improved significantly, the response latency in a passive avoidance response in senescence-accelerated mice (these animals showed a marked age-accelerated deterioration in learning tasks of passive avoidance), it was ineffective in normal-rate aging mice [77]. Consistently, two H_3 receptor antagonists, thioperamide and clobenpropit [126], lacked any procognitive effect in control animals [51], but fully reverted rats cognitive impairments, measured in a passive avoidance response and object recognition, caused by injection of scopolamine (0.2 mg/kg, i.p.) [51]. Similarly, FUB 181, another H_3 receptor antagonist [119], significantly ameliorated performances of scopolamine-impaired mice (0.5 mg/kg, i.p.) in the elevated plus-maze test [91]. Other studies, however, reported that administration of thioperamide or clobenpropit to scopolamine-impaired mice (1 mg/kg, i.p.) only attenuated scopolamine-induced impairments in the elevated plus-maze test and the step-through passive avoidance test, [80–82]. The use of a higher dose of scopolamine might explain this discrepancy.

Object recognition and passive avoidance responses might involve cholinergic neurons of the NBM, since both tasks were impaired by the cholinergic antagonist scopolamine [40,118]. In addition, axon-sparing ibotenic acid bilateral lesions of NBM neurons, including the cholinergic ones, which provide the innervation to the cortex and to the amygdala [39,78], disrupted the performance of rats in both tasks [9,37,95,106]. In fact, these paradigms serve to measure a form of episodic memory, possibly localized in the frontal cortex [53] and the amygdala [95]. Cognitive improvements produced by administration of H_3 receptor antagonists might be the result of relieving the inhibitory action on cortical acetylcholine by local H_3 receptors (see Section 4). A second potential mechanism that may have contributed to the effects of H_3 antagonists is the modulation of endogenous histamine release. Endogenous histamine exerted a tonic influence on cholinergic neurotransmission, enhancing cholinergic activity at the level of cholinergic cell bodies in the basal forebrain [7,22,83]. Thus, H_3 receptor antagonists, by increasing the release of endogenous histamine, may facilitate cholinergic activity in brain areas crucial for cognitive functions. However, a beneficial effect on a scopolamine-induced deficit is a concomitant observation, but does not prove in anyway that cholinergic neurons are involved. Reversal of impairments observed in the above mentioned studies may be also due to histamine direct effects on cognition, and/or to histaminergic modulation of any number of transmitter systems. More persuasive evidence of a close relationship between the cholinergic and histaminergic system in learning and memory is offered by the results of experiments with H_3 receptor agonists. Rat systemic pre-training administration of imetit and R-α-methylhistamine moderated potassium-evoked release of cortical ACh and impaired performance in object recognition and a passive avoidance response [14]. The disruption of the cortical cholinergic system may account for the cognitive impairments, since reduced availability of ACh in the synaptic cleft appeared related to cognitive deficits [111]. The lack of effectiveness of the same doses of imetit and R-α-methylhistamine when administered post-training, suggests that the H_3 receptor is involved in the acquisition but not the recall of this information [51]. However, ACh may control both acquisition and retention processes, since also post-training administration of scopolamine resulted in animals exhibiting significantly shorter escape latencies during a passive avoidance response, and spending similar amount of

time exploring new and familiar objects [51]. Therefore, the impairment of cognition by H_3 receptor agonists is unlikely attributable solely to the modulation of cortical acetylcholine. One might envisage mechanisms other than the cholinergic one, and the finding that R-α-methylhistamine improved rodent spatial learning and memory, assessed using a water maze [117], supports this contention. Spatial learning is a primary function of the rodent hippocampus [90], and the water maze test is exquisitely sensitive to hippocampal lesions [87], but H_3 receptor stimulation is expected to decrease hippocampal cholinergic activity [6,7].

6. Conclusions

For the complexity of the neuronal networks in the brain, it seems naïve to assume that only one neurotransmitter, namely ACh, regulates such a complex mechanism as learning and memory. Other neurotransmitter systems have been implicated in these processes. Decker and McGaugh [33] suggested a model in which, although ACh has a central role, interactions with other neurotransmitters, such as dopamine, GABA, noradrenaline, are essential for the formation of memory. This hypothesis is supported by several studies [18,52,112,121]. However, the modulation of the cholinergic pathways by other neurotransmitter systems, and the importance of the cholinergic system as a final effector in learning and memory, still needs to be defined. The aim of this review is to critically assess biochemical, electrophysiological and behavioral evidence of interactions between the cholinergic and the histaminergic systems, and to examine the possible role of such interactions in learning and memory. Biochemical as well as electrophysiological evidence indicates that ACh/histamine interactions appear to be complex and multifaceted (Fig. 1). Histamine activates cortical H_2 receptors, which are likely localized on GABA interneurons, and inhibits the release of cortical ACh through a GABAergic mechanism [14,48,50]. On the other hand, histaminergic projections to NBM exert a tonic influence on cortical cholinergic activity, depolarizing cholinergic cell bodies through activation of H_1 receptors [22,70], thus increasing ACh release from the cortex [22]. Conversely, activation of histaminergic neurons projecting to the basolateral nuclei of the amygdala inhibits the cholinergic tone in this area, and postsynaptic H_2 receptors seem responsible for this effect [101]. Finally, histamine effects on hippocampal cholinergic activity may involve actions at different anatomical locations. Local administration of histamine failed to affect ACh release from hippocampus, but MSA-DB endogenous histamine facilitates hippocampal cholinergic activity through activation of postsynaptic H_2 receptors possibly localized on septal cholinergic perikarya [6,7,83]. It is obvious that any attempt to strictly correlate physiological data with the outcome of behavioral tests is destined to fail. In several circumstances, though, one may envisage possible scenarios to account for the memory improving or impairing effects of histaminergic compounds in terms of modifications of ACh release. As an example, the depressant effect of H_1 antagonists on active avoidance response seems consistent with the action of H_1 receptors on NBM cholinergic neurons. A potential mechanism that may contribute to procognitive effects of H_3 antagonists is the modulation of endogenous histamine release, which is under an inhibitory feedback control by H_3 autoreceptors [5,84]. Endogenous histamine exerts a tonic influence on cholinergic neurotransmission, enhancing cholinergic activity at the level of cholinergic cell bodies in the NBM and MSA-DB [7,22,83]. Thus H_3 receptor antagonists, by increasing the release of endogenous histamine, may facilitate cholinergic activity in brain areas crucial for cognitive functions. It should be kept in mind, though, that the systemic administration of these histaminergic compounds can not account for a selective action on restricted brain regions. The scenario is certainly more complex; indeed, R-α-methylhistamine-induced improvement of rat performance in a water maze test [117] calls for a different explanation than simple ACh/histamine interactions. R-α-methylhistamine modulates, in addition to ACh, the release of either 5-HT or noradrenaline [114], and each of these transmitters has been shown to alter performance in a variety of cognitive tests [33]. Therefore, the possibility that at least some cognitive effects of histamine and histaminergic agents occur independently of ACh, cannot be excluded. It is also important to note that cognitive tasks don't necessarily imply that all behavioral changes should be interpreted in terms of learning and memory, since the link between the behavioral change and a cognitive process is not a one-to-one relationship. For instance, in a water maze test, escape latency may reflect not only the ability to learn the position of the hidden platform, but also exploratory aspects of the behavior.

While our understanding of the histaminergic system and its role in learning and memory is far from complete, we have progressed to the point where it is possible to address the importance of treatment strategies that, taking advantage of non-cholinergic drugs that potentiate cholinergic functions, may produce beneficial effects on disorders associated with impaired cholinergic functions, such as Alzheimer's disease [24]. This indirect approach appears preferable over cholinomimetic strategies. In fact, cholinergic drugs used in most clinical trials have resulted in greater stimulation of inhibitory autoreceptors either by increasing the half-life of acetylcholine in the synaptic cleft [28] or by directly activating these receptors due to the poor selec-

tivity of the agonists available [47]. Indirect stimulation of residual cholinergic neurons may be achieved with appropriate pharmacological intervention. Thus, H_3 receptor antagonists could correct the deficits resulting from cholinergic hypofunction, and provide a novel approach to improve cognitive deficits.

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Histamine and Seizures

Implications for the Treatment of Epilepsy

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Summary

Experimental studies have indicated that the central histaminergic neuron system plays an important role in the inhibition of seizures through stimulation of histamine H_1 receptors, especially in the developmental period. This has therapeutic implications for currently available drugs that act at histamine receptors. H_1 receptor antagonists, including classical antihistamines and anti-allergy drugs, occasionally induce convulsions in healthy children and patients with epilepsy. In particular, promethazine, carbinoxamine, mepyramine (pyrilamine) and ketotifen should be used with caution in these patients. These drugs are widely used as components of over-the-counter medications. The use of the *d*-chlorpheniramine (*d*-chlorpheniramine) activation study with EEG monitoring is useful for assessing the seizure susceptibility of patients who have had convulsions secondary to administration of H_1 receptor antagonists.

H_2 receptor antagonists have also occasionally been reported to induce convulsions in critically ill and polymedicated patients, and patients with chronic renal or hepatic failure. However, experimental findings have not been consistent with these clinical reports, such that the role of these receptors and their ligands in inducing seizures cannot be confirmed.

Recently, H_3 receptor antagonists, which enhance endogenous histamine release in the brain, have been demonstrated to have a potent anticonvulsant action. Therefore, these compounds may represent a new avenue for the development of antiepileptic drugs. Considering that H_3 receptor antagonists also induce arousal patterns on the EEG, it is possible that they will not be associated with the sedative effects of many conventional antiepileptic drugs.

1. Histamine in the CNS

1.1 Synthesis and Metabolism

Histamine, noradrenaline (norepinephrine), dopamine and serotonin (5-hydroxytryptamine; 5-HT) are biogenic amines found in the CNS, which are synthesised from precursor aromatic amino acids. The essential aromatic amino acids are: (i) tyrosine; (ii) tryptophan; and (iii) L-histidine. Histamine is synthesised from L-histidine, a reaction catalysed by histidine decarboxylase (see fig. 1).[1,2]

Histamine, like dopamine, cannot penetrate the blood-brain barrier.[3] However, L-histidine can easily enter the brain where it is converted to histamine. In the mammalian brain, histamine is inactivated by histamine *N*-methyltransferase to produce N^{π}-methyl-histamine.[1,2] This product is subsequ-

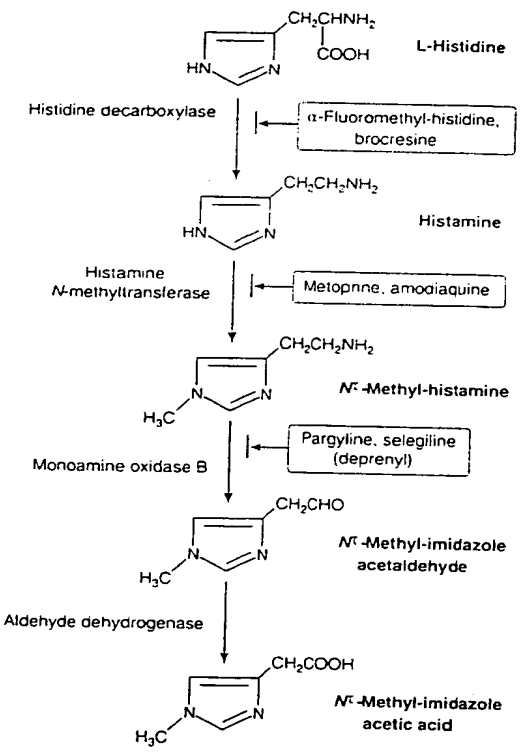

Fig. 1. Main metabolic pathway of histamine in the brain. Metabolic and catabolic enzymes are shown on the left and inhibitors of these enzymes in boxes on the right of the figure.

ently oxidised by monoamine oxidase and aldehyde dehydrogenase to N^{τ}-methyl-imidazole acetic acid (fig. 1).

1.2 Histamine Receptors

There are several subtypes of histamine receptors.[4] It is well known that histamine H_1 receptors are involved in the induction of allergic reactions and that H_2 receptors mediate the secretion of gastric acids, both effects occurring in peripheral tissues. H_1 and H_2 receptors also exist in the CNS and are located postsynaptically.[11,21] Recently, it has been suggested that presynaptic H_3 autoreceptors regulate the synthesis and release of neuronal histamine (fig. 2).[5,6]

1.3 Histaminergic Pathways and Function

In the 1970s, histamine was hypothesised to be a neurotransmitter or neuromodulator in the mammalian brain.[7-9] This was based on the finding that histamine and histidine decarboxylase are non-uniformly distributed in the brain. In the 1980s, Watanabe et al.,[10] Panula et al.[11] and Steinbusch et al.[12] immunohistochemically demonstrated independently the existence of histaminergic neurons in the brain, using antibodies against histidine decarboxylase and histamine as markers.

Histaminergic neurons in the tuberomammillary body nucleus of the posterior hypothalamus send efferent fibres of varicosity to almost all parts of the brain, from the olfactory bulb to the spinal cord (fig. 3). Consistent with this widespread projection, histamine affects a variety of brain activities such as:

- arousal state
- energy metabolism
- locomotor behaviour
- feeding behaviour
- the neuroendocrine system
- autonomic function
- regulation of pain.[12,13-15]

The extensive nature of histaminergic neurons has resulted in the suggestion that they are involved in maintaining homeostasis in the brain as a whole.[12,13,16]

1.4 Central Histaminergic System and Seizure Susceptibility

It has been suggested that biogenic amines such as noradrenaline and serotonin are involved in the inhibition of convulsions.[17-19] In 1976, Gerald and Richter[20] reported that some histamine H_1 antagonists could lower seizure susceptibility induced by electrical and drug insults in mice. More recently, Tuomisto and Tacke[21] showed that meto-

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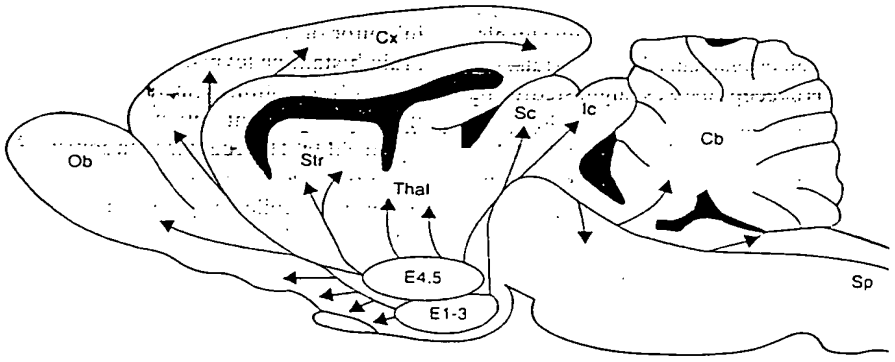

Fig. 3. A sagittal diagram of the histaminergic neuron system in the rat brain. Arrows indicate the fibre projections from cell bodies in the tuberomammillary body nucleus of the posterior hypothalamus, which are subdivided into 5 groups, E1 to 5. *Abbreviations:* Cb = cerebellum; Cx = cerebral cortex; Ic = inferior colliculus; Ob = olfactory bulb; Sc = superior colliculus; Sp = spinal cord; Str = striatum; Thal = thalamus.

rats.[124] H_3 receptor antagonists, which enhance endogenous histamine release in the brain, had an anticonvulsant action against electrically induced convulsions in mice.[125,126] Intracerebroventricular injection of histamine or a H_1 receptor agonist also decreased seizure susceptibility.[127]

However, it was reported that H_1 receptor antagonists did not affect the seizure susceptibility of mice at doses that specifically blocked the receptors.[128] Interestingly, in another study, these compounds did not affect the seizure susceptibility of electrically induced convulsions in 42-day-old (equivalent to adolescent age) mice,[129] but increased seizure susceptibility in 21-day-old (equivalent to preschool age) animals.[129] In addition, the effects of other histamine-related agents such as L-histidine and α-fluoromethyl-histidine were much greater in 21-day-old than in 42-day-old mice.[129] These findings suggest that the anticonvulsant action of central histaminergic neurons is physiologically important in the developmental period.

These experimental findings strongly suggest that endogenous histamine plays an important role in the inhibition of seizures through H_1 receptors,[12,14,15,20,22-27] and that this effect is particularly important in the developmental period.[129,130]

2. Histamine H_1 and H_2 Receptor Antagonists and Seizures

2.1 Histamine H_1 Receptor Antagonists

2.1.1 General Issues

H_1 receptor antagonists are widely used as nasal decongestants and anti-allergic agents.[131] It has been proven that these drugs have a wide margin of safety, although they often lead to drowsiness and impairments of performance.[132] These sedative adverse effects are considered to be due to the antagonism of H_1 receptors.[132]

H_1 receptor antagonists occasionally induce convulsions in healthy children and patients with epilepsy.[133-135] Tripelennamine and diphenhydramine have been shown to produce epileptic discharges on the EEG and clinical manifestations of psychomotor seizures in patients with epilepsy.[134] Diphenhydramine, methapyrilene, tripelennamine and mepyramine occasionally produce convulsions and lethal toxic encephalopathy with convulsions, especially in children under the age of 2 years.[135,136] Phenylpropanolamine is a component of some over-the-counter (OTC) preparations. Although it does not have effects at histamine receptors, it has been reported to induce unexpected convulsions when combined with antiasthmatic agents that act

at these receptors.[137,138] propanolamine and anti more proconvulsant ef were given alone. More reported that 2 infants age-dependent intractal treated for an allergic about 2 weeks.[139] Alt evaluate the relationsh West syndrome, it is v that the histaminergic n inhibition of convulsio

These drugs have se tions that lead to adver linergic and antidopami to their antihistaminerg pected proconvulsant initially believed to be linergic action.[133] Howe to be a more selective classical antihistamines. which has little antichol convulsions in epileptic indicate that the blocka responsible for the in healthy children and ep

Table I summarises t associated convulsions. ised into 2 groups: (i) c (ii) the new generation known as anti-allergic Clinicians tend to forg potent H_1 receptor bloc ketotifen has a much blocking action than *d*-pheniramine).[142] Altho nists are probably capal convulsions, particular to the use of promethazi mine and ketotifen.[143] possess more potent H reported more frequen than other H_1 antagonis

at these receptors.[37,38] The combination of phenylpropanolamine and antiasthmatic drugs resulted in more proconvulsant effects than when the drugs were given alone. More recently, Yasuhara et al.[39] reported that 2 infants developed West syndrome, age-dependent intractable epilepsy, after they were treated for an allergic disease with ketotifen for about 2 weeks.[39] Although it is impossible to evaluate the relationship between ketotifen and West syndrome, it is very indicative, considering that the histaminergic neuron system is involved in inhibition of convulsions.

These drugs have several pharmacological actions that lead to adverse effects, such as anticholinergic and antidopaminergic actions, in addition to their antihistaminergic action. Thus, the unexpected proconvulsant property of the drugs was initially believed to be mainly due to an anticholinergic action.[33] However, mepyramine is known to be a more selective H_1 antagonist than other classical antihistamines.[40] Furthermore, ketotifen, which has little anticholinergic action, also induces convulsions in epileptic children.[41] These reports indicate that the blockade of H_1 receptors may be responsible for the induction of convulsions in healthy children and epileptic patients.

Table I summarises the reports of antihistamine-associated convulsions. These drugs are categorised into 2 groups: (i) classical antihistamines and (ii) the new generation of H_1 antagonists that are known as anti-allergic agents, such as ketotifen. Clinicians tend to forget that the latter possess a potent H_1 receptor blocking action. For example, ketotifen has a much more potent H_1 receptor blocking action than *d*-chlorphenamine (*d*-chlorpheniramine).[42] Although all H_1 receptor antagonists are probably capable of inducing unexpected convulsions, particular caution should be applied to the use of promethazine, carbinoxamine, mepyramine and ketotifen.[43] This is because these drugs possess more potent H_1 blockade action and are reported more frequently to induce convulsions than other H_1 antagonists.

2.1.2 Febrile Convulsions

It is well known that the period of highest risk for convulsions in humans is childhood,[44] with the most frequent convulsions in children being febrile convulsions. Recently, it was reported that sympathomimetics[45] and potential proconvulsants, such as antihistamines,[43] should be used with caution in children, as they are a risk factor for febrile convulsions. These drugs are widely used as components of OTC medications to treat rhinorrhoea. According to our retrospective study,[43,45] use of antihistamines such as promethazine and carbinoxamine was one of the risk factors for febrile convulsions. As a result, antihistamines are best avoided in children of preschool age who have a past history of convulsions. Further prospective study is required to assess the relationship between antihistamines and febrile convulsions.

2.1.3 EEG Activation Studies

Antihistamines can be used during EEG monitoring. Diphenhydramine[46] and tripelennamine[47] were used to elicit epileptiform activities, having effects in epileptic patients but not in healthy individuals. Such 'activation' methods were reported to be very helpful in establishing a diagnosis of epilepsy.[47] However, these drugs are

Table I. Antihistamines that have been associated with convulsions

Reference	Year of study	Drug
Churchill & Gammon[34]	1949	Diphenhydramine Tripelennamine
Wyngaarden & Seevers[35]	1951	Diphenhydramine Methapyrilene Tripelennamine Pyranisamine Promethazine
Schwartz & Patterson[36]	1978	Mepyramine (pyrilamine)
Yokoyama et al.[41]	1993	Ketotifen
Ohsawa et al.[a]	1994	*d*-Chlorphenamine (*d*-chlorpheniramine)
Kon et al.[a]	1995	Hydroxyzine
Yokoyama et al.;[43] Takeuchi et al.[a]	1996	Clemastine
Yokoyama et al.;[43] Takeuchi et al.[a]	1996	Cyproheptadine

a Personal communication.

prine, an inhibitor of histamine *N*-methyltransferase which increases brain histamine levels, inhibits maximal electroshock seizures in rats.

Blockade of H_1 receptors was suggested to be important in lowering the seizure threshold in the pentetrazole (pentylenetetrazole) seizure model in mice.[122] L-histidine increases the levels of histamine in the brain and decreases susceptibility to electrically induced convulsions in mice.[123]

In contrast, α-fluoromethyl-histidine, a specific inhibitor of histidine decarboxylase that decreases brain histamine levels, has been shown to increase seizure susceptibility in an animal model.[123] Also, the anticonvulsant action of L-histidine was antagonised by H_1 receptor antagonists.[123]

Furthermore, regional brain histamine levels in genetically epilepsy-prone rats were found to be lower than those of genetically epilepsy-resistant

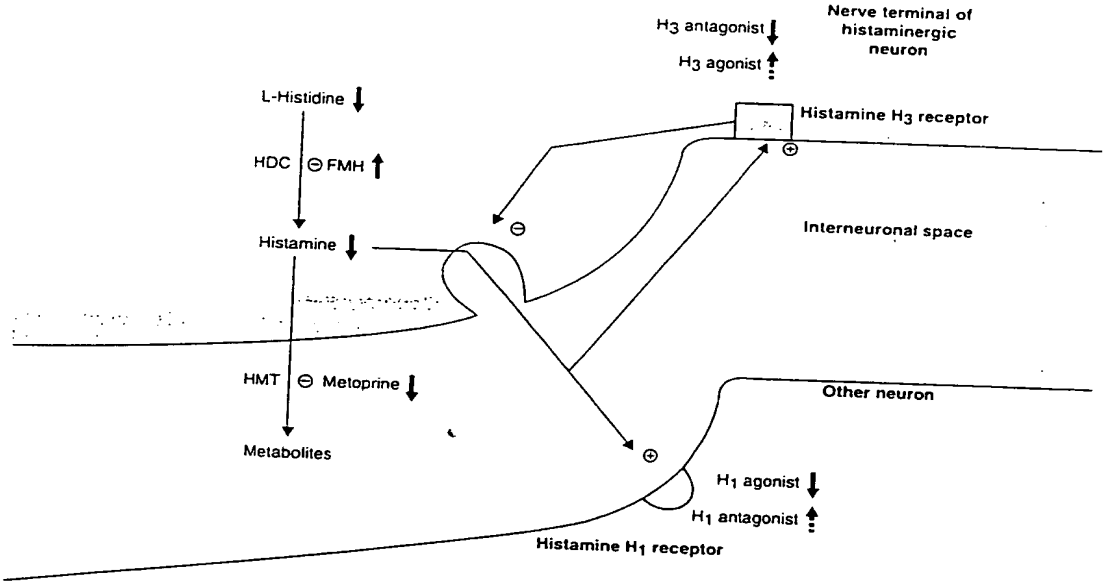

Fig. 2. Contribution of the histaminergic neuron system to the inhibition of seizures. L-Histidine (a precursor of histamine) and metoprine (an inhibitor of histamine metabolism) can increase brain histamine levels, resulting in a decrease in seizure susceptibility. In contrast, α-fluoromethyl-histidine (FMH, an inhibitor of histamine synthesis) decreases brain histamine levels and causes an increase in seizure susceptibility. Histamine and selective histamine H_1 receptor agonists decrease seizure susceptibility, whereas H_1 receptor antagonists increased it. H_3 receptor antagonists increase endogenous histamine release and decreased seizure susceptibility that is mediated via H_1 receptors. *Abbreviations and symbols:* HDC = histidine decarboxylase; HMT = histamine *N*-methyltransferase; ↑ indicates an effect resulting in an increase in seizure susceptibility; ↓ indicates an effect resulting in a decrease in seizure susceptibility; broken arrows indicate effects that are seen in developing mice only; + indicates stimulation; – indicates inhibition.

now considered to not be selective for H_1 receptors.

We reported on an activation study using *d*-chlorphenamine.[141,143] *d*-Chlorphenamine is more selective for H_1 receptors than other H_1 antagonists that are available for intravenous injection (in Japan). The procedure for the activation study has been described previously.[141] In short, patients are intravenously administrated *d*-chlorphenamine at a relatively low dose (5 mg/dose in adults). This dose is chosen because it has been proven to completely block central H_1 receptors, as assessed using positron emission tomography (PET) in humans.[148] The administration of *d*-chlorphenamine significantly increased EEG epileptic discharges in patients who had convulsions induced by H_1 antagonists. This activation study is useful for the follow-up of these epileptic patients.[141,143]

Epileptologists should be aware that drugs that block H_1 receptors occasionally produce iatrogenic seizures in children and patients with epilepsy.[131,33-38,41,43,45,49]

2.1.4 Other Issues

Histidinaemia is one of the inborn errors of amino acid metabolism and was first described by Ghadini et al.[150] It is caused by a defective decrease in the activity of histidine deaminase (histidase), a enzyme responsible for the metabolism of histidine in peripheral tissues. Brain histamine levels in patients with histidinaemia are much higher than in healthy individuals.[151] A follow-up study[152] revealed that the incidence of convulsions in patients with histidinaemia during childhood was 2% (35/1535). In the 1970s, surveys of the incidence of febrile convulsions in Japanese children undergoing a medical check at the age of 3 years old showed that the incidence of convulsions of a non-selected population was 7.5% (1340/17773).[143] Thus, the incidence of convulsions in patients with histidinaemia is significantly lower than that in healthy individuals. This finding is consistent with the hypothesis that histamine is an endogenous anticonvulsant.[121,23,24]

PET makes it possible to localise and quantify neurotransmitters and their receptors.[153] For example, opioid receptors[154] and benzodiazepine receptors[155] have been measured in patients who have epilepsy. The localisation and binding potentials of H_1 receptors were also measured in patients with localisation-related epilepsies using [^{11}C]doxepin PET (doxepin is a potent H_1 receptor antagonist, and the mapping of binding sites using doxepin PET was very similar to that of mepyramine PET[148]). The binding potential of H_1 receptors in epileptic foci was 10 to 50% higher than that of the unaffected side of the brain.[156] The increase in H_1 receptor binding in epileptic foci correlated inversely with a decrease in glucose metabolism.[157] This finding also directly indicates that the central histamine neuron system is involved in epilepsies that are mediated through H_1 receptors. However, whether these changes in H_1 receptors are causal in epilepsy or merely secondary (i.e. induced by epilepsy) is still unknown.

2.2 Histamine H_2 Receptor Antagonists

H_2 receptor antagonists are widely used in patients with gastrointestinal diseases. These drugs have also been reported occasionally to induce convulsions,[158-61] in conjunction with other neuropsychiatric symptoms such as confusion, hallucinations, delirium, agitation and excitation.[158-61] The effects usually occur in elderly, critically ill and polymedicated patients, or in patients with chronic renal or hepatic failure.[161] A study has reported that the adverse CNS effects of cimetidine are related to the blood concentration of metabolites, but not to that of the parent drug.[162]

Experimental studies confirmed that there are no relationships between convulsions and H_2 receptors.[122-23,26-28] H_2 receptor agonists and antagonists did not affect electrical- and drug-induced convulsions in mice and rats.[122-23,27-29] Therefore, it is difficult to ascertain the direct relationship between convulsions and the blockade of H_2 receptors.[161,62] Experimental studies suggest that H_2 receptors play no physiological role in the inhibition or induction of convulsions.

Nevertheless, clinicians should be aware of the neuropsychiatric adverse effects of H_2 receptor antagonists in certain patient populations.

Fig. 4. Chemical struc
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3. Histamine and Seizures

3.1 Histamine

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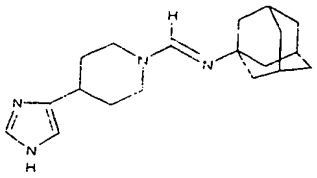

Fig. 4. Chemical structure of AQ-0145, a histamine H_3 receptor antagonist.

3. Histamine H_3 Receptors and Seizures

3.1 Histamine H_3 Receptors

Arrang et al.[15] reported evidence of a local feedback mechanism that regulates the release and synthesis of histamine through a presynaptic autoreceptor designated to be of the H_3 subtype (fig. 2). These authors also showed that *R*-α-methyl-histamine and thioperamide are specific agonists and antagonists of H_3 receptors, respectively.[16] *R*-α-methyl-histamine induces a decrease of histamine turnover, whereas thioperamide induces a prolonged increase of histamine turnover, indicated by changes in the content of histamine and N^{τ}-methyl-histamine in the cortex of rats.[163] Mochizuki et al.[164] observed an increase in endogenous histamine release by microdialysis techniques in the brain of rats after administration of thioperamide. They suggested that presynaptic control mediated through the H_3 autoreceptor is a major regulatory mechanism of histaminergic neuron activity.[16,64]

There have also been many indications of the interrelations between H_3 receptors and various other neurotransmitters and neuromodulators. It is suggested that H_3 receptors, as heteroreceptors, can modify the release of other neurotransmitters, such as noradrenaline,[165] serotonin[166] and acetylcholine.[167] On the other hand, glutamate, aspartate, acetylcholine and met-enkephalin stimulate histamine release, while γ-aminobutyric acid (GABA), substance P and adenosine inhibit its release.[168] The interactions of these transmitters with their respective receptors should be considered in future studies of the function of the histaminergic neuron system.

3.2 Histamine H_3 Ligands and Seizures

As described in sections 1.4 and 2.1, the stimulation of H_1 receptors is involved in the inhibition of seizures. Thus, it is possible that H_3 receptor antagonists could have anticonvulsive actions. This would occur via the induction of an increase in histamine release, with the released histamine stimulating H_1 receptors. Indeed, H_3 receptor antagonists such as thioperamide, clobenpropit and AQ-0145 (see fig. 4) decreased seizure susceptibility in a mouse model of electrically induced convulsions.[125,26,69] However, in another study, thioperamide did not affect the seizure susceptibility of mice.[122] These conflicting data resulted from the differing drug administration schedules used in these experiments. Thioperamide and clobenpropit showed anticonvulsive activity at 60, but not 30, minutes post-administration.[122,25,26] At a dose of 100 mg/kg, AQ-0145 inhibited electroshock-induced convulsions in 3 of 6 mice.[169] Thioperamide (30 mg/kg) and clobenpropit (3 to 10 mg/kg) also significantly inhibited maximal electroshock seizures (personal unpublished observations).

The anticonvulsive effects of H_3 receptor antagonists were much stronger than those of high doses of H_1 receptor agonists. Therefore, the interactions between H_3 receptors and other neurotransmitters and their receptors are suggested to be important in regulating seizure susceptibility,[115] and further studies on this aspect of H_3 receptor ligands is warranted.

Considering that H_3 receptor antagonists induce arousal patterns on the EEG, these drugs may represent a new generation of antiepileptic drugs that lack the sedative effects of conventional agents.[114,15] Research into the use of H_3 receptor antagonists is still very preliminary, but thioperamide, for example, is expected to be useful for the therapy of narcolepsy and dementia. Specific H_3 receptor ligands should be helpful in further studies and have recently become available.[170]

4. Conclusions

Histamine H_1 receptor antagonists occasionally induce convulsions in healthy children and patients with epilepsy. In particular, phenylpropanolamine, promethazine, carbinoxamine, mepyramine and ketotifen should be used with caution in these patients. The use of an activation study using *d*-chlorphenamine with EEG monitoring is useful in determining if these patients are susceptible to seizures induced by H_1 receptor antagonists. These clinical findings on the relationships between H_1 receptor antagonists and convulsions were confirmed in animal experiments.

H_2 receptor antagonists have also been reported occasionally to induce convulsions, which usually occur in conjunction with other neuropsychiatric symptoms such as confusion, hallucinations, delirium, agitation and excitation. These CNS adverse effects induced by H_2 receptor antagonists have occurred in critically ill and polymedicated patients, or patients with chronic renal or hepatic failure. However, there are no theoretical explanations for the relationship between the blockade of H_2 receptors and convulsions.

Several experimental studies have indicated that H_3 receptor antagonists show a potent anticonvulsive action against electrically induced convulsions in mice. Considering that these drugs induce arousal patterns on the EEG, they may develop into a new generation of nonsedating antiepileptic drugs. However, investigations into H_3 receptor antagonists are still in very preliminary stages.

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Diagnosis and Treatment of Toxoplasmosis of the CNS in Patients with AIDS

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Contents

Summary

Toxoplasmic encephalitis is a very common opportunistic infection in patients with AIDS (occurring in 5 to 15% of cases). It is the most frequent CNS manifestation in patients with previous *Toxoplasma* infection, as determined by the presence of specific antibodies.

A diagnosis of toxoplasmic encephalitis should be suspected in patients who present with clinical symptoms such as fever, headaches or any neurological abnormalities associated with the presence of intracerebral abscess on computerised tomography scans and/or magnetic resonance imaging. Early diagnosis leading to early treatment is the best prognostic factor for this treatable, but severe, disease.

The diagnosis is assessed by the response to therapy with a combination of pyrimethamine (50 mg/day) and sulfadiazine (4 g/day), which should lead to improvement within 5 to 10 days. The duration of acute therapy should be 3 to 6

REVIEW ARTICLE

Histamine and Betahistine in the Treatment of Vertigo

Elucidation of Mechanisms of Action

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Contents

Abstract

The aim of this review is to provide clinicians with a picture of the mechanisms by which: (i) histamine and histaminergic agonists act on the vestibular system both peripherally and centrally; and (ii) histaminergic agonists and antagonists interfere with the recovery process after peripheral vestibular lesion. We have focused on betahistine, a structural analogue of histamine with weak histamine

H_1 receptor agonist and more potent H_3 receptor antagonist properties, to review the currently available data on the role of the histaminergic system in the recovery process after peripheral vestibular deficits and the effects of histamine analogues in the clinical treatment of vertigo.

This review provides new insights into the basic mechanisms by which betahistine improves vestibular compensation in animal models of unilateral vestibular dysfunction, and elucidates particularly the mechanisms of action of this substance at the level of the CNS.

It is proposed that betahistine may reduce peripherally the asymmetric functioning of the sensory vestibular organs in addition to increasing vestibulocochlear blood flow by antagonising local H_3 heteroreceptors. Betahistine acts centrally by enhancing histamine synthesis within tuberomammillary nuclei of the posterior hypothalamus and histamine release within vestibular nuclei through antagonism of H_3 autoreceptors. This mechanism, together with less specific effects of betahistine on alertness regulation through cerebral H_1 receptors, should promote and facilitate central vestibular compensation.

Elucidation of the mechanisms of action of betahistine is of particular interest for the treatment of vestibular and cochlear disorders and vertigo.

Histamine is a biogenous amine of the brain that regulates various cerebral functions and induces many physiological effects. For example, the histaminergic pathways are involved in wakefulness, thermoregulation, pituitary function and cardiovascular regulation. Histamine modifies the neuronal activity of cortical and subcortical structures through neuromodulation and/or neurotransmission; therefore, histamine is generally considered to be a neuromodulatory transmitter.[1-6]

Antihistamines were the first drugs used in the treatment of allergic symptoms; they have since been found to be effective in the treatment of vertigo, probably due to a centrally induced depressive effect.[7-9] Betahistine, a structural analogue of histamine, has also been used as an antivertigo agent for over 20 years.[7] The vascular actions of histamine and histamine analogues have been known for a long time,[10] but the structural properties of these molecules and the role of pre- and post-synaptic histamine receptors in the neuromodulation of histaminergic transmission have only recently been elucidated. Other recent investigations have uncovered a vestibulo-hypothalamo-vestibular histaminergic pathway that may be involved in the physiology and pathophysiology of balance (see section 2.3). A series of animal experiments have further identified the mechanisms by which histamine analogues would act on the sensory organs (see section 3.2), the vestibular pathways and the histaminergic system (see section 3.3).

The aim of this article is to provide clinicians with a picture of the working mechanisms of histamine and betahistine or other histamine analogues at the peripheral and central levels. Animal studies have provided interesting insights into how structural analogues of histamine, such as betahistine, act on vestibular functions and how effective they are in assisting recovery from vestibular lesions and treating vertigo.

1. Pathophysiology of the Vestibular System

The peripheral vestibular receptors are made up of sensory cells that are sensitive to both angular accelerations (detected by the semicircular canals) and linear accelerations (detected by the otoliths of the utricule and saccule). The otolith system also detects changes in the gravito-inertial forces that result from the modification of head position in space. The peripheral labyrinth therefore constitutes a central inertial platform that detects the position and movement of the head.[11-13]

The anatomical and physiological properties of

the vestibular system have been previously described in full.[11] This system conveys an uninterrupted flow of both static and dynamic information to the brain. The vestibular neural messages travelling in the vestibular nerves are delivered to the four main nuclei (lateral, superior, medial and inferior) that form the central vestibular nuclei complexes. These nuclei constitute a centre where multiple modality sensory information (including visual, somatosensory and vestibular inputs) is integrated to form the basis for central reconstruction of head velocity signals in three-dimensional space by the brain.[11,14-16]

In addition to integrating spatial cues, the vestibular nuclei are involved in pre-motor and pre-perception functions. They are important in controlling posture and balance, head and eye stabilisation (i.e. gaze stabilisation in space), and in elaborating a central representation of body position and motion in space.

The control of balance and eye movement is partly subserved by second-order vestibular neurons with descending (vestibulospinal system) and ascending (vestibulo-ocular system) axonal projections. Branching vestibular neurons influencing both oculomotor and spinal neurons contribute to gaze stabilisation. Vestibulocortical pathways relaying through the subnuclei of the thalamus subserve more cognitive functions such as body orientation in space (subjective vertical), body perception (body scheme) and spatial memory. In turn, vestibular cortical areas (parieto-insular vestibular cortex, areas 2v and 3a) and subcortical (inferior olive, reticular formation, cerebellum) structures modulate vestibular nuclei activity and control vestibular functions.[17,18]

Consequently, a peripheral labyrinth deficit or a unilateral lesion of the vestibular nerve will cause a three-fold syndrome: related to posture (imbalance, falls, deviation of locomotor trajectory), oculomotor (spontaneous nystagmus, skew deviation, oscillopsia) and perception (vertigo, deviation of the perceived vertical). The deficits that form the vestibulospinal and vestibulo-ocular syndromes are common in a large number of species. Most of what is known about the perception syndrome derives from observations made in patients with vestibular dysfunction. As a general rule, all three syndromes include both static and dynamic components.[19,20] Figure 1 shows a diagrammatic representation of the vestibular pathways mediating vestibular responses in the roll plane and of their pathophysiology.

Compensation for these static and dynamic vestibular deficits constitutes a good model for the study of CNS plasticity and the investigation of adaptive capacities of the adult brain.[22] As a general rule, compensation for static and dynamic deficits are characterised by different time courses. Compensation for static deficits is extensive, often complete, and can take only days, but compensation for dynamic deficits can take several months and can be incomplete (sometimes minimal). The dissociation between static and dynamic deficits compensation can be partly explained by the intervention of different compensatory mechanisms in the vestibular compensation process. Pre-existing or new behavioural strategies, sensory (proprioception, vision) as well as functional (restoration of spontaneous and evoked vestibular nuclei activity) substitution mechanisms have been reported.[22] In the literature, few results have demonstrated that structural changes in the vestibular nuclei occur after vestibular lesion.[20] In contrast, many neurophysiological (changes in the static and dynamic properties of vestibular cells) and neurochemical (up- or down-regulation of inhibitory amino acids, acetylcholine, neuropeptides, and/or their receptors) modifications have been demonstrated experimentally.[20-24]

It is now well known that drug treatment can considerably modify the course of vestibular deficit compensation, either by accelerating (through treatment with stimulants) or by slowing down (with sedatives) the recovery process.[25] Vertigo and its disabling vegetative manifestations may be treated with antihistamines, acetylcholine receptor antagonists or sedatives, that all act centrally to depress vestibular responses and to suppress the symptoms of vertigo. This therapy may be admin-

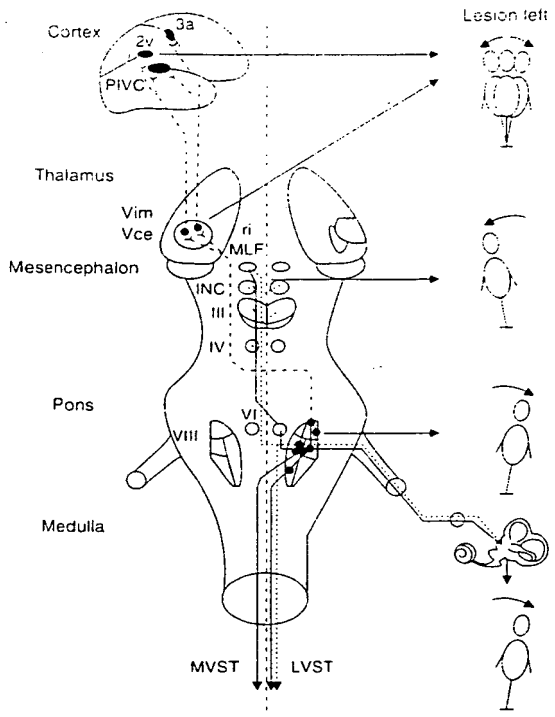

Fig. 1. Diagrammatic representation of the vestibular pathways that convey labyrinth sensory information to the four main vestibular nuclei of the brain stem via the VIIIth cranial nerve (VIII). The lateral and medial vestibulospinal tracts (LVST and MVST, respectively) are involved in head and body posture control. Ascending pathways in the medial longitudinal fasciculus (MLF) cross the midline and reach the interstitial nucleus of Cajal (INC) or the ocular motor nuclei [nucleus trochlearis (IV), nucleus oculomotorius (III), nucleus abducens (VI)]; these are involved in the oculomotor system and control the vestibulo-ocular reflex. Other crossed, ascending fibres relay through thalamus nuclei (Vim, Vce) and project into the parieto-insular vestibular cortex and other cortical areas receiving vestibular information (3a, 2v) that participate in self-motion perception, perception of the vertical and body scheme. The illustrations on the right show the direction of vestibular falls (or body tilts) and/or the perceived vertical in relation to the side and the level of the lesion (reproduced from Brandt and Dieterich,[21] with permission from Oxford University Press). **PIVC** = parieto-insular vestibular cortex; **ri** = rostral interstitial nucleus of the MLF; **Vce** = vestibular nuclei of the thalamus (central part of the ventral nuclei); **Vim** = vestibular nuclei of the thalamus (internal medullary part of the ventral nuclei).

istered to healthy individuals; however, it cannot be administered to patients with vestibular dysfunction for a long time without compromising the recovery process.[26,27] Although drugs that act as agonists or antagonists of histamine receptors have been used in the management of vestibular pathology,[7] very little was known until recently about the role of the histaminergic system in the control of vestibular functions and in the adaptive process after vestibular lesion.

2. Role of Histamine and Histamine Receptors in the Regulation of Vestibular Functions

Histamine is a neuromodulatory transmitter: the histaminergic system is widely distributed throughout various CNS structures.[2-5] Histamine and its three different categories of receptors have been found in the vestibular nuclei complexes, strongly suggesting that histamine plays a significant role in the regulation of vestibular functions.[1,6,28]

2.1 Central Histaminergic System

Biosynthesis of histamine within the CNS largely determines its cerebral distribution because it does not readily cross the blood-brain barrier (BBB). Histamine has a heterogenous distribution in the brain that parallels that of the enzyme that synthesises it, histidine decarboxylase (HDC).[29] Histamine in the brain is stored in both neuronal structures (50%), and non-neuronal (50%) structures such as the microvascular endothelium and the mast cells. The most important portion of histamine in the neuronal compartment is associated with the synaptic vesicles in the nerve endings, where it is synthesised. Three types of CNS histamine receptors have been identified in mammals: postsynaptic histamine H_1 and H_2 receptors[28,30] and presynaptic histamine H_3 receptors[31,32] (figure 2). H_1 receptors are coupled with phosphatidylinositol, H_2 receptors with adenylate cyclase and H_3 receptors with G-proteins. All three histaminergic receptors are metabotropic receptors, acting intracellularly through second messenger systems.

Using immunocytochemistry, histaminergic neurons were found to be exclusively located in the posterior hypothalamus, mainly in the tuberomammillary nucleus.[14,29,33] These neurons fire in a low, regular, spontaneous manner, and exhibit long-duration action potentials and slow axonal conduction. The histaminergic nerve endings project diffusely throughout the whole brain and show moderately sized swellings with a typical beaded appearance, which stain strongly. Such properties are typically seen in unmyelinated systems that exert neuromodulatory actions. We were the first to describe the projection of histaminergic nerve fibres to the four main vestibular nuclei in the cat brain, using an antibody directed against histamine.[16,34] Local variations were present, with more immunocytochemical staining in the superior and medial nuclei compared with the lateral and inferior nuclei.

Autoradiography using specific radioactive ligands made it possible to visualise the three types of histamine receptors. H_1 receptors are diffusely and heterogeneously distributed throughout the CNS.[30] They are blocked by antihistamines, for example, mepyramine.[7] There are only a few specific agonists of H_1 receptors, and these compounds do not readily cross the BBB.

H_2 receptors are also diffusely and heterogeneously distributed throughout the CNS.[28,35] Impromidine is a selective agonist of these receptors and cimetidine is a good, relatively selective antagonist. More potent and selective than cimetidine,

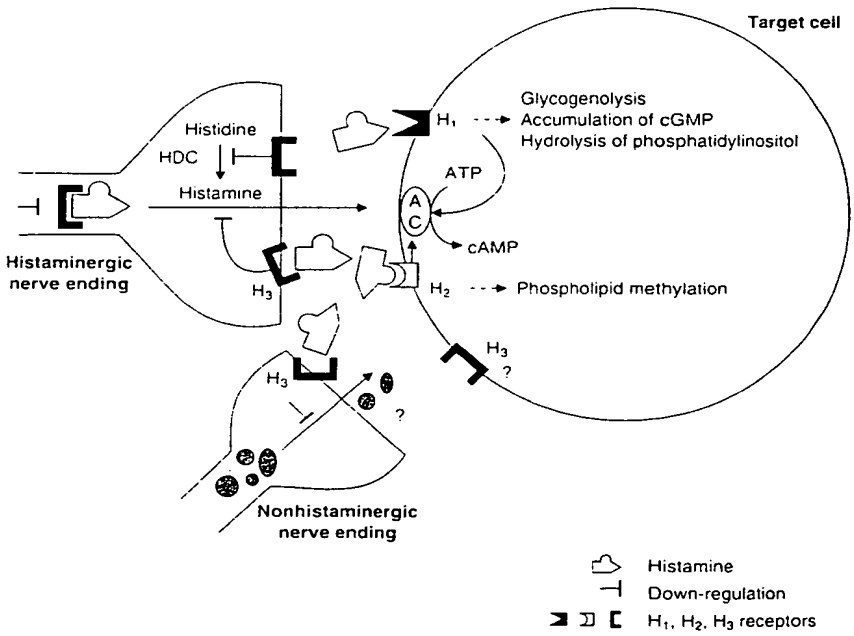

Fig. 2. A functional diagram of a hystaminergic synapse showing the three types of histamine receptor: postsynaptic histamine H_1 and H_2 receptors and presynaptic histamine H_3 receptors. Presynaptic H_3 receptors are located on histaminergic nerve endings and are known as autoreceptors; on nonhistaminergic endings they are called heteroreceptors (reproduced from Lacour,[6] with permission from Elsevier Paris). **AC** = adenylate cyclase; **ATP** = adenosine triphosphate; **cAMP** = cyclic adenosine monophosphate; **cGMP** = cyclic guanosine monophosphate; **HDC** = histidine decarboxylase.

H_2 receptor antagonists (zolantidine, famotidine, tiotidine) have been recently synthesised.

The same diffuse and heterogeneous distribution has been observed for H_3 receptors.[32] The standard agonist and antagonist for this receptor are α-methylhistamine and thioperamide, respectively. These substances regulate the synthesis and release of histamine by acting on the H_3 autoreceptors found on histaminergic nerve endings. Histamine and H_3 receptor agonists inhibit histamine turnover, whereas H_3 receptor antagonists promote the synthesis, turnover and release of histamine.[36] H_3 heteroreceptors are localised on non-histaminergic neuron endings, suggesting that the neuromodulatory action of histamine on its target cells may influence the synthesis and release of other neurotransmitters (figure 2).

It must be noted that all three categories of histamine receptors are present in the vestibular nuclei, at least in the guinea pig.[16,34,37,38]

2.2 Histamine and Neuromodulation of Vestibular Nuclei Neurons

Experimental data suggest that histamine modifies the central activity of the vestibular nuclei cells, and consequently, could affect the processing of sensory information in this structure and the control of vestibular functions. For example, hypothalamic histaminergic neurons project directly into the vestibular nuclei in the rat; it has been hypothesised that these afferents regulate motion sickness.[39]

Histamine modulation of second-order vestibular neuronal activity has been demonstrated in both *in vitro* and *in vivo* experiments; different effects were observed, depending on the preparation used. The neuronal spontaneous firing rate decreased after iontophoresis of histamine in cat vestibular nuclei in the first *in vivo* studies.[40] This inhibitory effect was confirmed by Kirsten and Sharma[41] who observed histamine-induced inhibitions of 78 and 68% in the medial nucleus neurons and the lateral nucleus cells, respectively. Facilitatory effects were seen in 9 and 27% of these respective nuclei neurons. The *in vitro* studies, carefully conducted on vestibular nucleus slices using a larger sample of units, clearly pointed to histamine-induced membrane depolarisation of the vestibular neurons. Histamine slightly increased the spontaneous firing rate of medial vestibular cells in both rats[42,43] and guinea pigs.[37] This effect was mimicked and inhibited by H_2 receptor agonists and antagonists, respectively, suggesting that the histamine depolarising effect is mediated through H_2 receptors.[37] Further experiments suggested that the H_1 receptors were also involved.[44]

The electrophysiological changes seen in the vestibular nuclei complexes have effects on function: behavioural modifications were recorded in animals receiving histamine receptor ligands infused chronically in the vestibular nuclei on one side.[38] Generally, the postural and oculomotor deficits observed were similar to those seen after unilateral vestibular lesion.[38] The vestibular postural syndrome was reproduced with an infusion of H_2 receptor antagonists or H_3 receptor agonists. The postural asymmetries were reversed with H_2 receptor agonists and H_3 receptor antagonists. Similar findings were reported for the oculomotor syndrome. It is generally assumed that these agonists and antagonists act by creating an imbalance in electrical activity between the homologous vestibular nuclei, similar to that produced by a unilateral vestibular lesion.

2.3 Evidence for a Histaminergic Vestibulo-Hypothalamo-Vestibular Loop

Unilateral stimulation of the labyrinth, which causes asymmetrical vestibular nuclei activity (electrophysiological recordings) and ocular movements typical of vestibular lesion-induced eye disturbances, has recently been observed to trigger a marked increase in histamine release in the posterior hypothalamus.[45] Roughly similar effects were demonstrated with microdialysis following both cold or warm caloric and electrical stimulation of the labyrinth. In addition, the effects of a more natural and bilateral vestibular stimulation were investigated in rats by placing them in a centrifuge and subjecting them to rotations which

can cause the behavioural symptoms of motion sickness.[39] Histamine levels increased in the hypothalamus and pons regions of healthy, intact rats; no effect was seen in rats with both labyrinths destroyed or treated with α-methylhistidine, an inhibitor of the enzyme that synthesises histamine.

Expression of the Fos protein was used as a neuronal marker to functionally map brain stem activity after stimulation or lesion of the vestibular system. In rats submitted to increased gravito-inertial forces (hypergravity) in a centrifuge, the spatial pattern of Fos induction involved the vestibular nuclei and associated structures (inferior olive, autonomic nuclei) and the tuberomammillary of the posterior hypothalamus.[46] We found a roughly similar pattern of Fos expression in unilateral vestibular neurectomised cats.[47]

Together these data clearly demonstrate that increased or imbalanced activity in the vestibular nuclei complexes leads to the activation of the central histaminergic system. This activation occurs via a vestibulo-hypothalamo-vestibular loop which may be important in the control of vestibular functions and in the adaptive response to vestibular lesion and environmental changes.

3. Histamine and the Histamine Analogue Betahistine in Peripheral and Central Vestibular Disorders

3.1 Receptor Binding Affinity

Betahistine (N-alpha-methyl-2-pyridylethylamine) is a structural analogue of histamine with similar pharmacological properties. The effects of betahistine on histamine brain receptors have been studied in several biological models.[48] It has been shown that betahistine is a weak H_1 receptor agonist, a more potent H_3 receptor antagonist and has negligible H_2 receptor activity. Affinity for receptors of other neurotransmitters demonstrated, but affinity for autonomic α_2 receptors is suspected (see section 3.2).[49,50]

Several studies have clearly demonstrated that betahistine is an H_1 receptor agonist:

- Betahistine and mepyramine (an H_1 receptor antagonist) compete for binding to cerebellar cell membranes in guinea pigs.[51,52]
- Both betahistine and histamine produce concentration-dependent hydrolysis of labelled glycogen in murine brain cortex sections, but the peak glycogenolytic effect of betahistine is weaker than that of histamine.[48]
- In a dose-dependent manner, betahistine stimulates the cyclic AMP accumulation induced by impromidine (a selective H_2 receptor agonist) but at a 20% lower level than histamine.[48]
- The binding of mepyramine to the murine brain cortex *in vivo* is inhibited following the systemic administration of betahistine, therefore betahistine must be able to cross the BBB.[48] Following systemic administration, the intracerebral concentration of betahistine was equivalent to half of the concentration of the compound in blood.[52] However, the affinity of betahistine for the H_1 receptor is weak: its action at H_1 receptors was only observed at dosages 100-fold higher than therapeutic dosages.[48]

Other studies have demonstrated that betahistine is also an H_3 receptor antagonist:

- In a dose-dependent manner, betahistine blocks the suppression of synaptic release of histamine and other transmitters induced by exogenous histamine via the H_3 autoreceptor.[31]
- The concentration of betahistine required for this effect *in vivo* is considerably lower than that necessary to produce excitation of the cerebral H_1 and/or H_2 receptors.[48]

The affinity of betahistine for the H_3 receptor in the brain, as measured by the dissociation constant, is less than that of thioperamide, another H_3 receptor antagonist.[36,37,48]

3.2 Peripheral Mechanisms and Sites of Action of Betahistine

3.2.1 Vascular Mechanisms in the Inner Ear

The increase in cochlear blood flow following systemic administration of betahistine was first reported many years ago.[53-55] The mechanism of action by which betahistine alters this microcircu-

lation was studied more recently by Laurikainen et al.[49,50] using laser doppler flowmetry. The increase in cochlear blood flow caused by betahistine is inhibited by atropine (an acetylcholine receptor antagonist), idazoxan (an autonomic α_2-receptor antagonist) or thioperamide (an H_3 receptor antagonist), but not modified by cimetidine (an H_2 receptor antagonist) or α-methylhistamine (an H_3 receptor agonist). Therefore, betahistine antagonises the presynaptic H_3 heteroreceptors of the cochleovestibular vascular system via the activation of autonomic α_2-receptors.[49,50] This effect is only achieved by administering betahistine through the vascular route (catheterisation of the antero-inferior cerebellar artery) and not by direct application to the inner ear through the round window.[49]

Peripheral vasodilation has also been observed with systemic administration of betahistine, as evidenced by a decrease in blood pressure which is blocked by prior administration of promethazine. However, the betahistine dose required to produce the cochleovestibular effect was four-fold lower than that required to produce the systemic effect (0.5 *vs* 2.1 mg/kg to produce 50% of the maximum effect).[49]

It appears that the betahistine-induced increase in cochlear blood flow is associated with an increase in vascular conductivity and a decrease in systemic blood pressure (figure 3),[49] and may result primarily from vasodilation of the anterior inferior cerebellar artery. This effect appears to involve H_1 receptors, presynaptic H_3 heteroreceptors and autonomic α_2-receptors, at least in the inner ear of the guinea pig.[50]

In contrast, an infusion of betahistine induced a decrease in systemic blood pressure and blood flow in the vestibular apparatus, followed by a sustained increase in vestibular blood flow while the blood pressure rapidly returned to normal.[56] Regulation of the vestibular blood flow in the posterior semicircular canal ampulla has been observed in guinea pigs, and can be influenced by various factors including betahistine.[56-58] The vascular control of the vestibule may be different to that of the cochlea and requires further investigation.

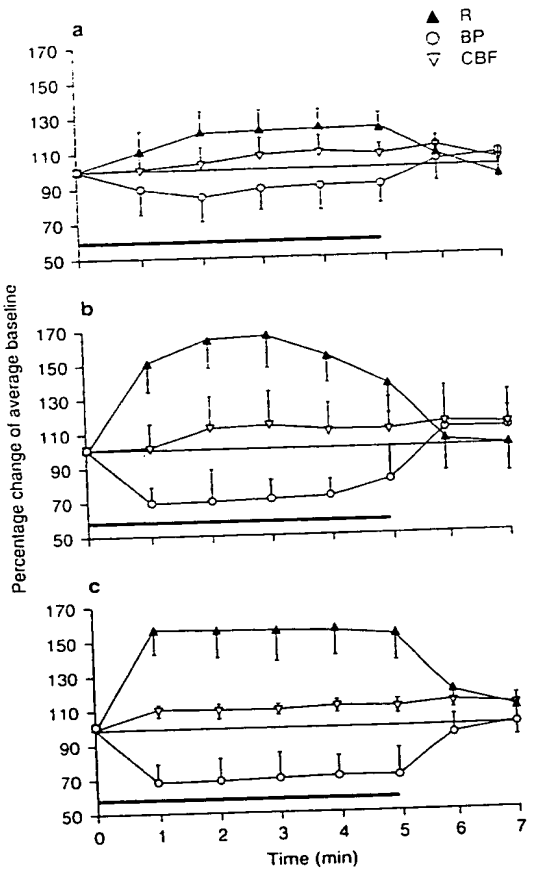

Fig. 3. Effect of betahistine (a) 0.9 mg/kg, (b) 1.8 mg/kg or (c) 3.6 mg/kg, administrated intravenously (shown by shaded bar) on cochlear blood flow (CBF), blood pressure (BP) and vascular conductivity (R) [= CBF/BP] in rats (reproduced from Laurikainen et al.,[49] with permission Lippincott Williams & Wilkins).

3.2.2 Physiological Mechanisms on Inner Ear Sensory Organs

The effect of betahistine on isolated preparations of frog posterior semicircular canal was recently examined.[59] The drug was administered via the endolymphatic or perilymphatic fluid, and its effects on spontaneous or evoked resting discharges of ampullar receptors were measured. No effect was observed with endolymphatic administration. In contrast, perilymphatic administration

of betahistine decreased the resting discharge of the ampullar receptor to a large extent, but had a minimal effect on the mechanically evoked response. Drug concentrations as low as 10^{-7} mol/L were sufficient to reduce the resting firing rate, whereas inhibition of the evoked activity was obtained at doses as high as 10^{-2} mol/L (figure 4).

The effect of betahistine on vestibular receptors in the frog suggests that the drug does not act on the mechanosensitive channels of the hair cells, but on the basolateral membranes of the sensory cells, dark cells and/or afferent nerve terminals. The mechanism of action may involve histamine receptors in the peripheral vestibular system,[60] although this remains to be confirmed. Whatever the mechanism of action involved, it could contribute to the antivertigo effects of betahistine.

3.3 Central Mechanisms and Sites of Action of Betahistine

3.3.1 Facilitation of the Recovery Process After Vestibular Lesion: Behavioural Data

Without administration of betahistine, compensation for static and dynamic balance deficits developed within 40 days of unilateral vestibular neurectomy in a cat model. Posturolocomotor function was totally restored within 5 to 6 weeks,[61,62] as evidenced by normalisation of the support surface area (static index) and the locomotor performance on a rotating beam (dynamic index) [figure 5]. The restoration of the spontaneous activity of vestibular neurons located in the ipsilateral lateral nucleus occurred over a similar time period (unpublished observations).[6] Oral administration of high dosages of betahistine (50 or 100 mg/kg/day) over a time period of 3 to 4 weeks reduced the time to recovery by 2 weeks for both static and kinetic function compared with no treatment or placebo.[61]

We assumed therefore that betahistine enhances the behavioural recovery of vestibular functions in these animals, but how the drug achieves this was not clear.[61] Since the drug is a structural analogue of histamine, an H_3 receptor antagonist and a partial H_1 receptor agonist (section 3.1), we hypothesised that it increases histamine turnover (synthesis) and release. This hypothesis was tested using immunocytochemical techniques (section 3.3.2).

3.3.2 Interaction with the Histaminergic System: Immunocytochemical Data

After unilateral vestibular neurectomy and/or betahistine treatment, histamine immunoreactivity

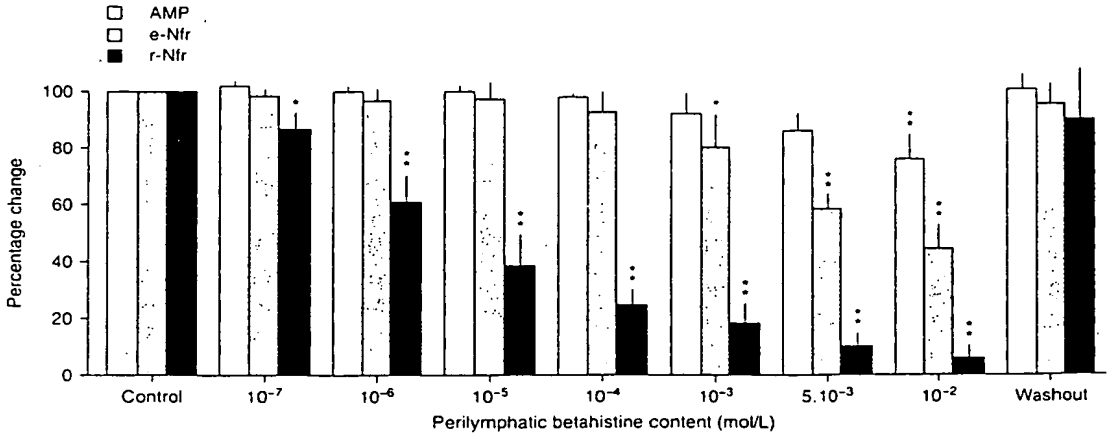

Fig. 4. Effect of betahistine on vestibular receptor discharge [ampullar microphonic potential (AMP), evoked nerve firing rate (e-Nfr) and resting nerve firing rate (r-Nfr)] in isolated preparations from frogs as a function of perilymphatic betahistine content (reproduced from Botta et al.,[59] with permission from Scandinavian University Press). * $p < 0.05$, ** $p < 0.001$ *vs* control.

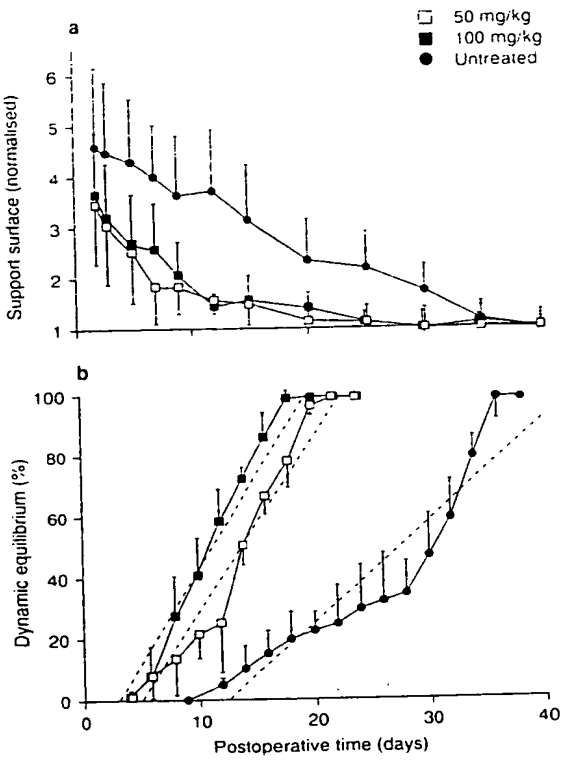

Fig. 5. Effect of betahistine on posturolocomotor function recovery (reproduced from Tighilet et al.,[61] with permission from IOS Press). Recovery profiles are presented for both (a) static and (b) dynamic balance functions in unilateral vestibular neurectomised cats which received betahistine 50 or 100 mg/kg/day postoperatively for 1 month or remained untreated. Units for support surface are normalised with respect to the controls for which the measurements are in cm^2.

changes were investigated in both the tuberomammillary nuclei (which contain the histaminergic neurons) and the vestibular nuclei (where histamine nerve terminals are found[16,34]) of cats. Immunohistochemical and light microscopy quantification techniques were used.

A unilateral vestibular lesion induced a large bilateral decrease in histamine immunoreactivity in the vestibular nuclei and a smaller reduction in the posterior hypothalamus (tuberomammillary) in cats at both 1 week (acute) and 3 weeks (compensated) after neurectomy.[62] Cats without a vestibular lesion that were treated with betahistine exhibited a nearly total lack of histamine staining in these structures, a result that was reproduced in cats treated with thioperamide, an H_3 receptor antagonist (figure 6). The combination of vestibular lesion and betahistine treatment also resulted in a histamine immunoreactivity level that was close to zero.

These results strongly suggested that betahistine increases histamine turnover and synthesis, consistent with its pharmacological properties. Low levels of histamine immunoreactivity in the vestibular and tuberomammillary nuclei were attributed to depletion of histamine in synaptic vesicles and nerve terminals, the result of increased histamine synthesis and release. This, in turn, would be due to the blocking effect of histamine on the presynaptic H_3 receptors. Additional experiments were needed to confirm that these are the central mechanisms of action of betahistine.

3.3.3 Interaction with the Histaminergic System: New Molecular Findings

The effects of both betahistine treatment and vestibular lesion on the histaminergic system were investigated using *in situ* hybridisation and autoradiography techniques. Hybridisation was used to analyse changes in messenger RNA (mRNA) coding for HDC in the tuberomammillary nuclei; autoradiography was used performed with a tritiated selective H_3 receptor agonist (^{3}H-N-α-methylhistamine; ^{3}H-NαMH) and used to investigate the density of H_3 binding sites (which mediate histamine autoinhibition) in the tuberomammillary and vestibular nuclei.

Quantification of both HDC mRNA and H_3 receptor binding site densities showed asymmetrical changes after unilateral vestibular neurectomy in the cat, with an up-regulation of HDC mRNA and a decrease in ^{3}H-NαMH labelling on the lesioned side (unpublished observations). In contrast, treatment with betahistine induced symmetrical changes in intact animals, with an up-regulation of HDC mRNA and a reduction in ^{3}H-NαMH labelling on both sides (unpublished observations). Figure 7 il-

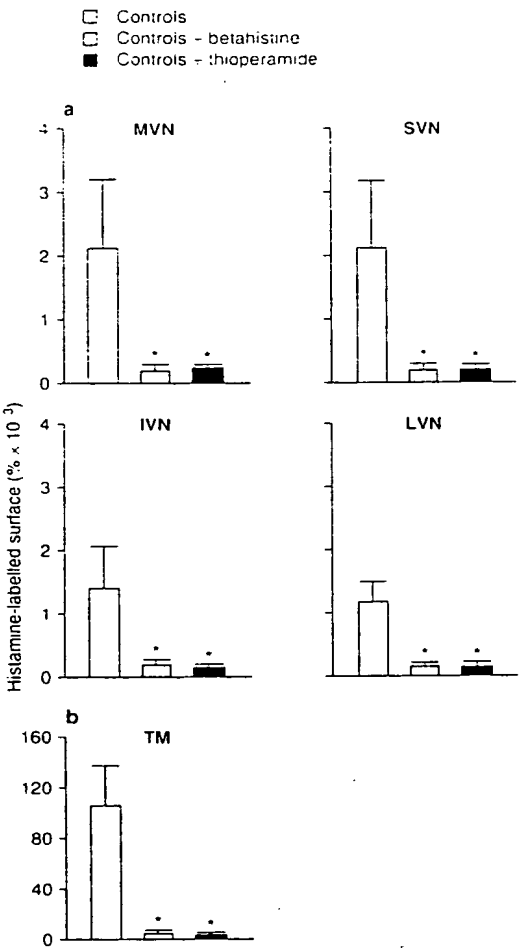

Fig. 6. Quantitative evaluation of the effects of histaminergic substances on histamine immunoreactivity in (a) the vestibular nuclei and (b) the posterior hypothalamus in cats treated with betahistine or thioperamide, or control cats (reprinted from the European Journal of Pharmacology, Tighilet and Lacour,[62] with permission from Elsevier Science). Results were obtained for the medial (MVN), superior (SVN), inferior (IVN) and lateral (LVN) vestibular nuclei and tuberomammillary nuclei (TM). * $p < 0.001$ *vs* controls.

lustrates the increase in expression of HDC mRNA in the posterior hypothalamus of cats that received betahistine for 1 or 3 weeks compared with control cats.

These results clearly demonstrate that betahistine increases the synthesis of histamine by the histaminergic neurons of the tuberomammillary nuclei by blocking the presynaptic H_3 autoreceptors. Histamine release is then enhanced in several CNS structures, including the tuberomammillary and the vestibular nuclei. Unilateral vestibular lesion would produce similar but asymmetrical effects through a different mechanism of action: the activation of a vestibulo-hypothalamo-vestibular loop.

3.3.4 Effects on Vestibular Nuclei Neurons and Interaction with Histamine

Betahistine exerts a neuromodulatory action on central vestibular neuronal activity, which is characterised by changes in excitability both *in vivo* and *in vitro*.

Betahistine inhibits the response of neurons in the medial vestibular nucleus to rotatory stimulation in the horizontal plane[63] and the spontaneous firing rate of polysynaptic neurons in the lateral vestibular nucleus *in vivo*.[64] However, few neurons were recorded in these studies and the nature of the cells (for example, type I, type II, inhibitory or excitatory neurons) was not determined; therefore, it is not possible to claim that betahistine inhibits the spontaneous or the evoked activity of second-order vestibular neurons.

The effect of betahistine on vestibular neuronal activity was more carefully studied *in vitro*[44] by recording the tonic activity of medial vestibular nucleus neurons from slices of brainstem preparations taken from young adult rats. However, the actions of H_3 receptors are generally not detectable in slices. Histamine had an excitatory effect on these neurons via the H_1 and H_2 receptors. When applied alone, low doses of betahistine had no excitatory effect on eight neurons activated by histamine, and high concentrations (300 to 1000 μmol/L) induced weak excitation (figure 8). Conversely, the excitatory effect of histamine was reduced with simultaneous application of betahistine. This effect was dependent on betahistine concentration and could not be reproduced when the neuron was stimulated by serotonin. The betahistine-induced reduction of the histamine-induced

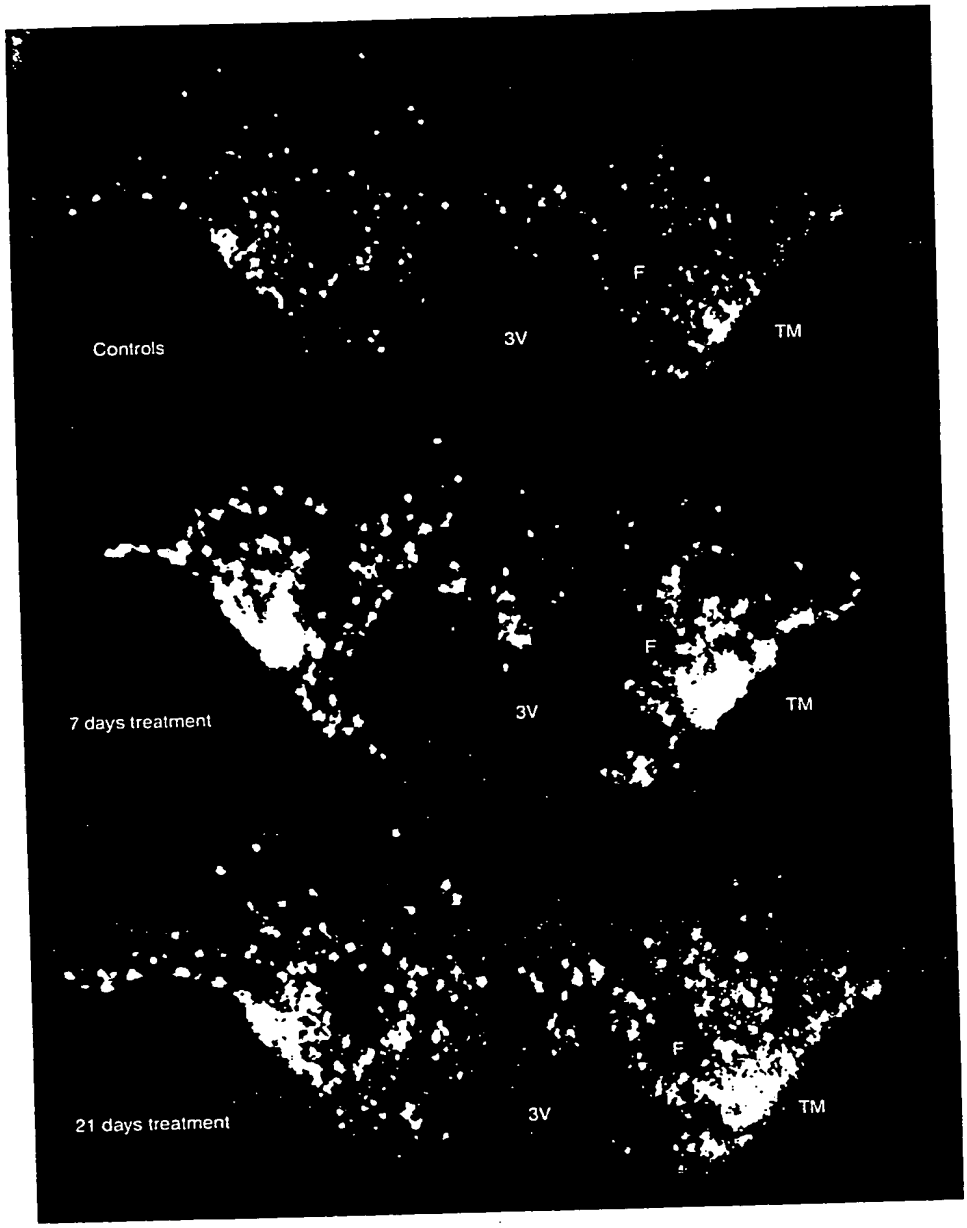

Fig. 7. Quantitative evaluation of the expression of messenger RNA coding for histidine decarboxylase, the enzyme that synthesises histamine, in the tuberomammillary nuclei (TM) of cats treated with betahistine 50 mg/kg/day for 1 or 3 weeks or control cats (unpublished observations). 3V = third ventricle; F = fornix.

excitatory response of vestibular cells can be explained by the partial agonistic properties of betahistine at the H_1 receptor level and competition between endogenous histamine and betahistine for H_1 receptors.

These hypotheses were supported by our recent molecular investigation (unpublished observations). We found that the combination of vestibular lesion and treatment with betahistine led to the down-regulation of HDC mRNA expression at 1 week after treatment and up-regulation at 3 weeks. These observations can again be explained by competition between betahistine and histamine for the H_3 receptor. Indeed, changes in the binding of ^{3}H-NαMH to H_3 receptors, characterised by a reduction in the density of binding sites at 1 week post-lesion and an increase at 3 weeks' post-lesion, strongly suggests a competitive interaction between histamine (the release of which is increased in the vestibular nuclei during the acute stage) and betahistine (which predominates over histamine during the compensated stage). These results do not exclude another mechanism hypothesis involving plasticity at the cellular level: long term treatment with betahistine could lead to a permanent H_3 receptor blockage which could then produce a reactive or compensatory up-regulation of this receptor subtype.

3.4 Effect of Betahistine on Cortical Activity: Vigilance and Psychomotor Performance

3.4.1 Nystagmus and Vestibulo-Ocular Responses

In patients with paroxysmal vertigo of 3 months' duration who were receiving betahistine 16, 32 or 64mg three times daily, the duration and amplitude of the nystagmus response induced by different stimulations were evaluated in a double-blind, placebo-controlled study.[65] Following low-frequency sinusoidal rotation in darkness or high-frequency passive rotation of the head, the amplitude of the induced nystagmus decreased by 20 and 50% with betahistine 16 and 32mg, respectively. Reduction in the amplitude of nystagmus was not observed when visuovestibular interaction was allowed in the presence of a visual target in the dark. Such results suggest that betahistine exerts its action on the vestibular nuclei.

In patients with vertigo who responded to betahistine treatment, betahistine was not found to have a dose-dependent effect on the vestibulo-ocular reflex, as it does in healthy individuals.[66] Indeed, the duration of the nystagmus response induced in ten healthy individuals submitted to the swinging seat test was decreased by 38 and 59% with betahistine 16 and 32mg, respectively. The effect peaked at 3 to 4 hours after betahistine administration and no adverse effects were observed.[67]

3.4.2 Vestibular Evoked Potentials

In a preliminary double-blind study, betahistine produced different effects on vestibular evoked potentials in healthy individuals and patients with peripheral vestibular disorders.[66] In patients with vertigo, the pathologically delayed mean latency of vestibular evoked potentials was normalised following administration of betahistine 48mg three times daily. A return towards control values occurred shortly after drug administration; the effect peaked at 3 hours postdose. Conversely, a progressive delay was introduced to the mean latency of vestibular evoked potentials following administration of the same dosage of betahistine to healthy individuals.

These findings suggest that the effect of betahistine on cortical activity may differ depending on whether recipients have vertigo. Since vertigo is a result, at least in part, of an imbalance of activity between the vestibular nuclei complexes on each side, the difference in cortical activity could be due to the activation of the vestibulo-hypothalamo-vestibular loop. As discussed in sections 2.3 and 3.3, stimulation of this pathway induces histamine release in the vestibular nuclei, and betahistine and endogenous histamine compete for binding to histamine receptors at the cellular level.

3.4.3 Psychomotor Performance and Driving Tasks

A double-blind, placebo-controlled study investigated the effects of high dosages of betahistine (72mg three times daily for 3 days) or the antipsychotic drug prochlorperazine (5mg three times daily) on the psychomotor performances of healthy

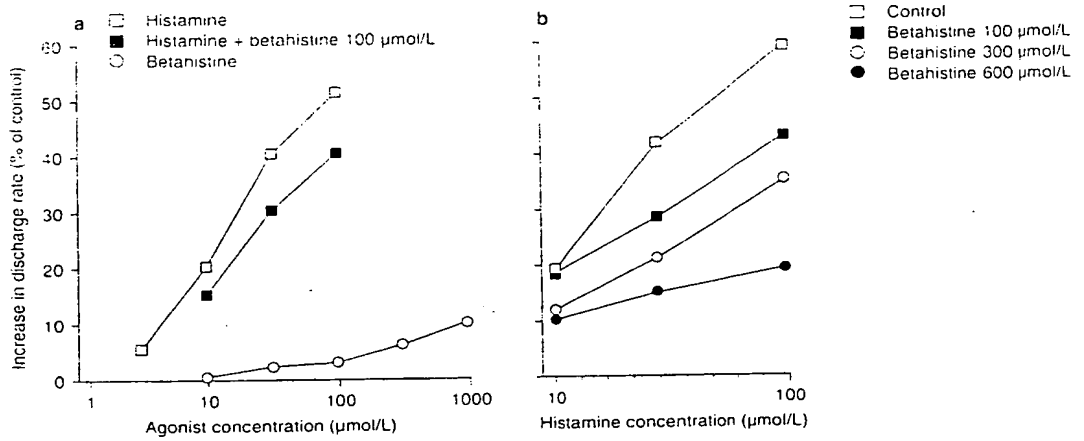

Fig. 8. (a) Dose-response curves showing the effects of histamine and betahistine applied separately or in combination on the firing rate of a rat medial vestibular nucleus (MVN) neuron. (b) Effects of different concentrations of betahistine on the response of an MVN cell to histamine (two drugs applied concurrently) [reproduced from Wang and Dutia,[44] with permission from Springer-Verlag].

volunteers who participated in a driving test.[68] The tests were carried out at baseline and at 1 week later (following drug administration) and examined reaction time and kinetic visual acuity (psychomotor performance) and weaving and gap estimation (driving tasks).

No sedative effect was observed following administration of betahistine. Individuals who received betahistine performed similarly to those who received placebo or who did not receive any treatment. In contrast, psychomotor and driving performances were markedly altered following administration of prochlorperazine. This drug relieves vertigo, nausea and vomiting of vestibular origin. It has a dual antagonistic action in the CNS, blocking both dopamine D_2 receptors and H_1 receptors. Since dopamine is known to facilitate cortical arousal, the alterations in behaviour observed after administration of prochlorperazine should directly result from the blockade of dopamine receptors. The blockade of H_1 receptors may contribute indirectly to the behavioural alterations because histamine induces excitatory effects on the neuronal activity of cortical and subcortical structures by activating H_1 receptors.[33]

4. How Does the Interaction Between Betahistine and Histamine Improve the Vestibular Compensation Process and Vertigo?

There is a growing body of experimental data that describes how histamine and betahistine act on the body at the peripheral and central levels. It has been discovered that:

- Histamine is involved in the regulation of vestibular functions (section 2).
- Histamine enhances the restoration of vestibular function after a deficit (through adaptation) by activating a vestibulo-hypothalamo-vestibular loop (section 2.3).
- Betahistine also has beneficial effects on this recovery process and interacts with histamine at the cellular level (section 3.3).
- Betahistine acts on peripheral and central targets, through H_1 and H_3 receptors (sections 3.2 and 3.3).

- In the CNS, betahistine increases histamine synthesis and release by blocking H_3 autoreceptors (section 3.3).

The main property previously ascribed to histamine, its vasodilative action on blood vessels,[1-6,10] is also a property of betahistine. The vascular effects of betahistine have been fully described and it is now well established that this drug acts on the vestibulocochlear vasculature, increasing blood flow and vascular conductivity and decreasing systemic blood pressure through H_3 heteroreceptors and H_1 receptors and autonomic α_2-receptors.[49,50,53-58]

Histamine and structural analogues of histamine like betahistine are also effective in the treatment of vestibular disturbances unrelated to vascular insufficiency, clearly indicating that there are other, probably more active, mechanisms through which they contribute to antivertigo effects. These can be subdivided into nonspecific and specific working mechanisms, acting either peripherally or centrally.

Betahistine has no adverse effects and is devoid of sedative action (section 3.4.3).[7,10,65,67,68] In addition, it increases histamine synthesis and release, enhancing alertness and vigilance.[33] This nonspecific arousal mechanism can play a significant role in the recovery process in patients with vestibular lesion. It is well known that sedative drugs slow down functional recovery, whereas stimulants accelerate recovery and increase sensorimotor activity.[25] The latter action is considered to be a key element in vestibular compensation in both animal models[69] and humans.[22]

The specific mechanisms of action of betahistine have been elucidated in animal models using autoradiography, *in situ* hybridisation and electrophysiology (section 3.3).

In the periphery, it was found that betahistine decreased the ampullar receptor resting discharge, an effect that could be mediated by histamine receptors in the peripheral vestibular sensory system. This peripheral vestibular mechanism may also be involved in reducing the asymmetrical functioning of the bilateral sensory receptors, but is not completely understood.

The most important discoveries involving the mechanisms of betahistine concern the action of the drug on the histaminergic system and histamine receptors. Betahistine increases histamine turnover (synthesis) and release by blocking H_3 autoreceptors. Histamine has depolarising effects on second-order vestibular cells in vestibular nuclei complexes, exerted through H_1 receptors. This central vestibular mechanism is probably involved in reducing asymmetrical activity of the vestibular nuclei neurons on both sides. The restoration of balanced electrical activity between the homologous vestibular nuclei, as demonstrated *in vivo* and in conscious animal models,[70] is central to the compensation for static vestibular deficits.[22] Additionally, there is a close correlation between behavioural adaptation and central re-equilibration of vestibular nuclei activity.[22,70]

The interaction between exogenous betahistine and endogenous histamine released in response to betahistine or a vestibular lesion (via the activation of a vestibulo-hypothalamo-vestibular loop) gives a complex picture of the central action of the drug. The competition between histamine and betahistine for binding to H_1 and H_3 receptors varies depending on the dose administered, the time period of administration and the stage of the vestibular lesion (acute or compensated). The mechanisms behind this variation, the neuromodulating action of histamine on the synthesis and release of other neurotransmitters (e.g. acetylcholine) and the betahistine-induced plasticity of H_3 receptors remain unresolved and require further investigation.

5. Conclusions and Clinical Implications

Several of the mechanisms by which betahistine acts on the vestibular system have been identified.

In the peripheral vestibular organs, betahistine decreases the ampullar receptor resting discharge, which reduces the asymmetrical functioning of the sensory vestibular organs. Betahistine also increases vestibulocochlear blood flow by antagonising local H_3 heteroreceptors, thereby protecting the vestibulocochlear sensory organ.

In the central vestibular nuclei, betahistine enhances histamine synthesis within tuberomammillary nuclei and histamine release within vestibular nuclei through antagonism of H_3 autoreceptors. This mechanism should promote and facilitate central vestibular compensation.

Additionally, betahistine regulates alertness, affecting the cortical and subcortical structures of the CNS through a partial agonist effect on cerebral H_1 receptors.

Most of the mechanisms by which histamine and betahistine act on the vestibular system have been elucidated. The clinical implications of these mechanisms in the vestibular system (peripherally and centrally) are of interest in the treatment of vestibular and cochlear dysfunctions and vertigo. Betahistine is widely used in the treatment of Meniere's disease and vertigo, and may yet have other therapeutic applications in inner ear disorders such as tinnitus, sudden hearing loss or noise-induced hearing loss.

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Neurochemical and Behavioral Effects of Ciproxifan, A Potent Histamine H_3-Receptor Antagonist

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ABSTRACT

Ciproxifan, *i.e.*, cyclopropyl-(4-(3-1*H*-imidazol-4-yl)propyloxy) phenyl) ketone, belongs to a novel chemical series of histamine H_3-receptor antagonists. *In vitro*, it behaved as a competitive antagonist at the H_3 autoreceptor controlling [^{3}H]histamine release from synaptosomes and displayed similar K_i values (0.5–1.9 nM) at the H_3 receptor controlling the electrically-induced contraction of guinea pig ileum or at the brain H_3 receptor labeled with [^{125}I]iodoproxyfan. Ciproxifan displayed at least 3-orders of magnitude lower potency at various aminergic receptors studied in functional or binding tests. *In vivo*, measurement of drug plasma levels, using a novel radioreceptor assay in mice receiving ciproxifan p.o. or i.v., led to an oral bioavailability ratio of 62%. Oral administration of ciproxifan to mice enhanced by ~100% histamine turnover rate and steady state level of *tele*-methylhistamine with an ED_{50} of 0.14 mg/kg. Ciproxifan reversed the H_3-receptor agonist induced enhancement of water consumption in rats with and ID_{50} of 0.09 ± 0.04 mg/kg, i.p. In cats, ciproxifan (0.15–2 mg/kg, p.o.) induced marked signs of neocortical electroencephalogram activation manifested by enhanced fast-rhythms density and an almost total waking state. In rats, ciproxifan enhanced attention as evaluated in the five-choice task performed using a short stimulus duration. Ciproxifan appears to be an orally bioavailable, extremely potent and selective H_3-receptor antagonist whose vigilance- and attention-promoting effects are promising for therapeutic applications in aging disorders.

HA is a cerebral neurotransmitter exerting its actions on target cells via three classes of molecularly and/or pharmacologically well defined receptors designated H_1, H_2 and H_3 (reviewed by Hill *et al.*, 1997; Schwartz *et al.*, 1991, 1995). The H_3 receptor is a presynaptic receptor regulating the synthesis and/or release of HA itself (Arrang *et al.*, 1983) as well as a variety of other aminergic or peptidergic neurotransmitters (reviewed by Schlicker *et al.*, 1994). It was initially defined by the design of two selective ligands: (R)α-MeHA, a full agonist, and thioperamide, an antagonist with nanomolar potency ($K_i \cong 4$ nM). Thioperamide has become the prototypical H_3-receptor antagonist, used in a large number of neurochemical, electrophysiological and behavioral studies because it is one of the few agents able to markedly enhance cerebral histaminergic transmissions *in vivo* via a selective mechanism. In agreement, few other actions of thioperamide were described, *e.g.*, inhibition of P450 cytochromes (La Bella *et al.*, 1992) and 5-HT_3-receptor blockade (Leurs *et al.*, 1995), which require higher drug concentrations than H_3-receptor blockade and are therefore not relevant for *in vivo* studies.

Nevertheless thioperamide has several drawbacks: 1) its *in vivo* potency is rather low compared with its *in vitro* potency, suggesting that drug bioavailability, particularly its brain penetration, is restricted, 2) more importantly it displays a distinct liver toxicity on repeated administration which has prevented it being submitted to human clinical trials.

Because H_3-receptor antagonists represent a novel class of agents with potentially interesting therapeutic applications, namely in psychiatry (Schwartz *et al.*, 1995) sustained efforts have been devoted to the design of drugs more potent and safer than thioperamide (reviewed by Stark *et al.*, 1996b).

As with thioperamide, all highly effective compounds obtained so far contain a monosubstituted imidazole ring, but

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ABBREVIATIONS: HA, histamine; t-MeHA, *tele*-methylhistamine; (*R*)α-MeHA, (*R*)α-methylhistamine; AUC, area under the curve; C_{max}, maximal concentration; W, wakefulness; S1, light slow wave sleep; S2, deep slow wave sleep; PS, paradoxical sleep; EEG, electroencephalogram.

the thiourea moiety of the latter, to which hepatotoxicity might be attributable, is replaced by numerous polar functionalities such as amine or carbamate, etc.

Recently, we have designed 3-(1*H*-imidazol-4-yl)propanol derivatives as a novel series of potent *in vitro* H_3-receptor antagonists with a high capacity for oral absorption and brain penetration in some compounds (Stark *et al.*, 1996a; Hüls *et al.*, 1996). In these novel chemical classes of compounds, we have recently identified [^{125}I]iodoproxyfan, *i.e.*, [^{125}I]3-(1*H*-imidazol-4-yl)propyl-(4-iodophenyl)methyl ether as a new probe for a sensitive assay and localization of the H_3 receptor in brain (Ligneau *et al.*, 1994).

We describe the biological properties of ciproxifan (fig. 1), a highly potent and selective H_3-receptor antagonist belonging to this novel chemical class of compounds which suggest its potential therapeutic interest as a waking and procognitive agent.

Materials and Methods

[^{3}H]Histamine release from synaptosomes. [^{3}H]HA release experiments were performed according to Garbarg *et al.* (1992). Briefly, a crude synaptosomal preparation from rat cerebral cortex was preincubated for 30 min with [^{3}H]L-histidine (0.4 μM) at 37°C. After extensive washing synaptosomes were resuspended in fresh 2 mM K^+-Krebs-Ringer's medium and in the presence of the appropriate drugs. After 5-min incubation synaptosomes were depolarized bringing the K^+-concentration to 30 mM for 2 min. Incubations were ended by a rapid centrifugation and [^{3}H]HA levels in the supernatant were determined after an ion-exchange chromatography purification. Release was expressed as the percent fraction of total [^{3}H]HA initially present in the synaptosomal preparation. Typically total [^{3}H]HA represented about 3,500 dpm/mg protein and total radioactivity about 100,000 dpm/mg protein in the test tube.

Assay of t-MeHA in brain. Male Swiss mice (18–20 g) or male Wistar rats (140–160 g) (Iffa-Credo, L'Arbresle, France) were fasted for 24 hr before p.o. administration. After treatment animals were killed by decapitation, the brain was dissected out and homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 N). The clear supernatant obtained after centrifugation (2000 × *g*, 30 min, + 4°C) was stored at −20°C before measuring the t-MeHA level by radioimmunoassay as described (Garbarg *et al.*, 1992). Changes were evaluated statistically by the Student's *t* test.

[^{125}I]Iodoproxyfan binding assays. The procedure for binding assays to rat striatal brain membranes was that described by Ligneau *et al.* (1994). Aliquots of membrane suspension [100 μl containing 15 to 20 μg of protein determined according to Lowry *et al.* (1951) using bovine serum albumin as standard] were incubated for 60 min at 25°C with 25 pM [^{125}I]iodoproxyfan (K_d = 65 ± 4 pM) alone or together with competing drugs dissolved to give a final volume of 200 μl in a phosphate buffer medium (Na_2HPO_4/KH_2PO_4 50 mM, pH 6.8). Incubations performed in triplicate were stopped by four additions of 5 ml ice-cold medium followed by rapid filtration through glass microfiber filters (GF/B, Whatman, Maidstone, U.K.) presoaked in a 0.3% polyethylene imine ice-cold buffer. Radioactivity trapped on filters was measured on a gamma counter.

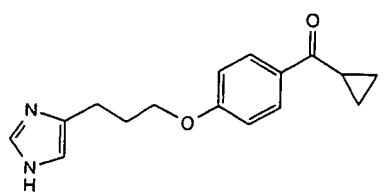

Fig. 1. Chemical structure of ciproxifan, *i.e.*, cyclopropyl-(4-(3-(1*H*-imidazol-4-yl)propyloxy)phenyl) ketone.

Histamine H_1 receptor assay on guinea pig ileum. The procedure used was that described by Pertz and Elz (1995).

Histamine H_2 receptor assay on guinea pig right atrium. The procedure used was that described by Pertz and Elz (1995).

Muscarinic M_3 receptor assay on guinea pig ileum. The procedure used was that described by Pertz and Elz (1995).

Adrenergic α_{1D} receptor assay on rat aorta. The procedure used was that described by Hirschfeld *et al.* (1992).

Adrenergic β_1 receptor assay on guinea pig right atrium. The procedure used was that described by Pertz and Elz (1995).

Serotoninergic 5-HT_{1B} receptor assay on guinea pig iliac artery. The procedure used was that described by Pertz (1993).

Serotoninergic 5-HT_{2A} receptor assay on rat tail artery. The procedure used was that described by Pertz and Elz (1995).

Serotoninergic 5-HT_3 receptor assay on guinea pig ileum. The procedure used was that described by Elz and Keller (1995).

Serotoninergic 5-HT_4 receptor assay on rat esophagus. The procedure used was that described by Elz and Keller (1995).

Histamine H_3 receptor assay on guinea pig ileum. The procedure used was that described by Ligneau *et al.* (1994). Briefly, longitudinal muscle strips from guinea pig small intestine were dissected out and incubated in a gassed O_2/CO_2 (95%/5%) modified Krebs-Ringer's bicarbonate medium at +37°C in presence of 1 μM mepyramine to block the H_1 receptor. After equilibration, contractile activity under stimulation (rectangular pulses of 15 V, 0.5 msec, 0.1 Hz) was recorded. Concentration-response curves of the effect of (*R*)α-MeHA alone or together with the antagonist were established.

Radioreceptor assay of H_3-receptor ligands in serum. Male Swiss mice (18–20 g, Iffa-Credo, L'Arbresle, France) were fasted for 24 hr before ciproxifan administration. At various times thereafter, mice were decapitated. Blood was collected at +4°C, serum collected after centrifugation (100 × *g*, 10 min, +4°C), and stored at −20°C. Ciproxifan levels were measured with the following radioreceptor assay derived from the [^{3}H](*R*)α-MeHA binding assay (Garbarg *et al.*, 1992).

Male Wistar rats (160–180 g, Iffa-Credo, L'Arbresle, France) were decapitated and the brain was removed immediately. The cerebral cortex was dissected out and homogenized in 10 volumes (w/v) of ice-cold 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5 using a Polytron. After a centrifugation (140 × *g*, 10 min, +4°C), the supernatant was recentrifuged (23,000 × *g*, 10 min, +4°C). The last pellet was washed superficially with and then resuspended in fresh ice-cold phosphate buffer to constitute the membrane fraction used for the binding assay. One-ml aliquots of the membrane suspension containing 300 to 330 μg of protein [determined according to Lowry *et al.* (1951) using bovine serum albumin as standard] were incubated for 60 min at +25°C with 1 nM of [^{3}H](*R*)α-MeHA alone or together with different concentrations of ciproxifan in diluted serum of ciproxifan-free mice (standardization curve) or with diluted serum samples of ciproxifan-treated mice. Specific binding was defined as that inhibited by 3 μM thioperamide. Incubations were performed in triplicate and stopped by four additions of 5 ml of ice-cold phosphate buffer followed by rapid filtration through glass microfiber filters (GF/C, Whatman, Maidstone, U.K.) presoaked in 0.3% polyethylene imine ice-cold phosphate buffer. Radioactivity trapped on filters was measured by liquid scintillation spectrometry (Wallac 1410, EG&G, Evry, France). The standardization curves were established using a one-site competition model (GraphPad Prism, San Diego, CA) and H_3-receptor ligand concentrations in serum were calculated using these curves and expressed as ciproxifan concentrations. In the conditions retained, the detection limit of the radioreceptor assay corresponded to a concentration of 20 nM ciproxifan in serum. Changes in H_3-receptor ligand concentrations in serum with time were analyzed using either a point to point nonlinear model or the two phase exponential decay analysis model (GraphPad Prism).

(*R*)α-MeHA-induced water consumption in rats. The procedure used was that described by Clapham and Kilpatrick (1993) with slight modifications. Briefly, male Lister hooded rats (280–320 g,

Charles River, St Aubin lès Elbeuf, France), housed in cages of 10, were allowed free access to food and water. Experiments were carried out between 10 A.M. and 1 P.M. Drugs in 0.9% NaCl solution were administered i.p., each rat receiving two injections [vehicle or (*R*)α-MeHA 10 mg/kg/p.o., and vehicle or ciproxifan] of 250 μl each. After drug treatments, rats were returned to their home cage with food (standard pellet) and without water for 30 min. Then they were placed in individual cages with only a water supply and 10 min later, the amount of water consumed was recorded by weighing. Statistical evaluation of results was performed by Student's *t* test.

Analysis of neocortical EEG power spectral density and sleep-wake in the cat. Cats, a species in which the role of the H_3 receptor in sleep-wake control has been demonstrated, were used in this experiment (Lin *et al.*, 1990). Briefly, five adult cats of both sexes weighing 2.7 to 3.8 kg were chronically implanted, under pentobarbital anesthesia (25 mg/kg, i.v.), with electrodes for polygraphic recordings of neocortical and hippocampal EEG, ponto-geniculo-occipital activity, electromyogram and electrooculogram. In addition, a thermistor (10 K3 MCD2, Betatherm, 10 KΩ at 25°C, outer diameter of 0.45 mm) was placed in the caudate nucleus to record brain temperature. After a recovery period of 7 days the cats were housed in a sound-attenuated and dimly illuminated cage set at 24 to 26°C and fed daily at 6 P.M. with a normal standard diet. Polygraphic recordings were performed for 4 days to obtain the basic qualitative and quantitative parameters of the sleep-wake cycle.

Ciproxifan at doses of 0 (placebo), 0.15, 0.3, 0.7 and 2 mg/kg was administrated orally at 11 A.M. Subsequent polygraphic recordings were made for at least 24 hr. They were then scored minute by minute according to previously described criteria (Lin *et al.*, 1990) for wakefulness (W), light slow wave sleep (S1), deep slow wave sleep (S2) and paradoxical sleep (PS). In some animals, neocortical EEG signals from the first 6 hr after placebo or drug administration were digitized at a sample rate of 128 Hz and computed on a CED 1401 Plus (Cambridge Electronic Design, Cambridge, U.K.). The power spectral density was averaged over 30-sec epochs for the frequency range of 0.25 to 50 Hz by a Fast Fourier Transform routine using the CED program Spike2 and correlated with sleep-wake stages.

Five-choice task in rats. Male Lister hooded rats (Olac, Bicester, U.K.) were housed in pairs in a temperature-controlled (21°C) room that was illuminated in accordance with an alternating 12-hr light/dark cycle. Rats were food deprived and maintained at 85% of their free-feeding weight (MRC Diet 41B laboratory food) throughout the experiment while water was available *ad libitum*.

The test apparatus and procedure were as described in detail by Muir *et al.* (1994). Rats were trained to discriminate a brief visual stimulus presented in one of five spatial location during 30-min sessions. Rats initiated a trial by opening the magazine panel. After a 5-sec inter-trial interval a light at the rear of one of the five apertures was illuminated for 0.5 sec. Correct responses to the stimulus location were rewarded with the delivery of food pellets. Having obtained stable performance on the task (>80% correct responses), attentional demand of the task was increased by reducing the stimulus duration to 0.25 sec in certain drug sessions as described previously (Muir *et al.*, 1994, 1995). Drugs or the vehicle (saline) were administered i.p. 1 hr before the test session (0.5- or 0.25-sec stimulus duration) according to a Latin square design. A rest day followed by a baseline day separated each drug test day.

Analysis of data. Maximal effects, ED_{50}, EC_{50} and IC_{50} values were determined using an iterative computer least-squares method derived from that of Parker and Waud (1971) with the following nonlinear regression: effect of the drug = ([maximal effect of the drug].[drug dose-or-concentration])/([drug dose-or-concentration] + (ED_{50} or EC_{50} or IC_{50})).

K_i values of H_3-receptor antagonists are calculated from their IC_{50} values, assuming a competitive antagonism and by using the relationship (Cheng and Prussoff, 1973):

$$K_i = IC_{50}/(1+(S/K_d))$$

where S and K_d represent respectively either the concentration and the dissociation constant of the radioligand in binding experiments, or the concentration of HA and its EC_{50} in [^{3}H]HA release experiments. When a fixed concentration of ciproxifan, tested as an antagonist, was added to increasing imetit concentrations, the K_i value of ciproxifan was calculated from the following equation (Cheng and Prussoff, 1973)

$$K_i = I/[(EC_{50}'/EC_{50})-1]$$

where EC_{50} and EC_{50}' are the imetit concentrations required to obtain half-maximal inhibition of release in the absence and presence of ciproxifan respectively, and I is the ciproxifan concentration.

Radiochemicals and drugs. [^{125}I]Iodoproxyfan and [^{3}H](*R*)α-MeHA (specific activities at reference date of 2000 and 38.0 Ci/mmol, respectively) were from Amersham (Amersham, U.K.). All drug doses are expressed as free base of compound. Administration to animals was performed with drug preparation in 1% methylcellulose for the oral route, in 0.9% NaCl for i.v. and i.p. routes. The drugs and their sources were as follows: ciproxifan [cyclopropyl-(4-(3-(1*H*-imidazol-4-yl)propyloxy)phenyl) ketone] synthesis was described in the patent application (Schwartz *et al.*, 1996); thioperamide and (*R*)α-MeHA (Laboratoire Bioprojet, Paris, France); carboperamide [1-(heptanoyl)-4-(1*H*-imidazol-4-yl)piperidine] was from M. Robba (University of Caen, France); clobenpropit was provided by H. Timmerman (Vrije Universiteit, Amsterdam, The Netherlands); iodoproxyfan was synthesized at the Freie Universität Berlin; imetit was synthesized by S. Athmani (University College, London, U.K.). All other chemicals were obtained from commercial sources and were of the highest purity available.

Results

Effects of ciproxifan on H_3-receptor functional models in vitro. The H_3-receptor agonist imetit inhibited the [^{3}H]HA release with a maximal effect of 54 ± 2%. Pharmacological parameters were estimated using the H_3-receptor controlled inhibition curve of the [^{3}H]HA release. Imetit concentration required for half inhibitory effect of its own maximal effect was 1.6 ± 0.3 nM and the corresponding pseudo-Hill coefficient (n_H) of this concentration response curve was close to unity (0.85 ± 0.15). Ciproxifan (15 nM) induced a parallel rightward shift of the concentration-response curve for imetit and tended by itself to increase (~20%) the K^+-induced [^{3}H]HA release; the antagonism developed by ciproxifan was entirely surmounted in the presence of the highest imetit concentrations tested (fig. 2). The half-maximal inhibitory concentration for imetit in the presence of ciproxifan (estimated considering the total H_3-receptor controlled inhibition curve) was 12.8 ± 1.8 nM, leading to an apparent K_i value of 1.9 ± 0.3 nM for the antagonist.

Histamine (1 μM) inhibited its own release by 55 ± 2%, and a series of H_3-receptor antagonists progressively reversed this response with n_H coefficients close to unity (fig. 3). IC_{50} values (nM) of the various compounds were 9.2 ± 1.8 (ciproxifan), 75 ± 19 (thioperamide), 377 ± 38 (carboperamide), 11 ± 3 (clobenpropit) and 99 ± 5 (iodoproxyfan) leading to K_i values reported in table 1, assuming a competitive inhibition.

Ciproxifan (3–300 nM) competitively antagonized the (*R*)α-MeHA induced relaxation of the electrically stimulated guinea pig ileum longitudinal muscle (Ligneau *et al.*, 1994) without significantly affecting the maximum response of the H_3-receptor agonist in the absence of ciproxifan [91 ± 5 (3 nM ciproxifan, n = 2), 100 ± 4 (10 nM, n = 6), 110 ± 10 (30 nM,

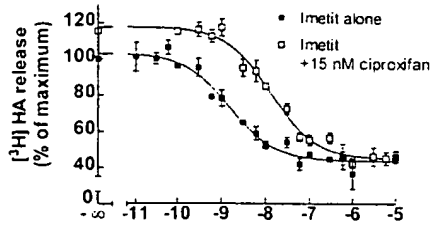

Fig. 2. Effect of ciproxifan on the inhibition by imetit of the K^+-induced [^{3}H]HA release from synaptosomes of rat cerebral cortex. Synaptosomes preincubated with [^{3}H]L-histidine were incubated for 5 min in the presence of imetit in increasing concentrations, alone or together with ciproxifan at a fixed (15 nM) concentration. They were subsequently depolarized for 2 min in the presence of 30 mM K^+ (final concentration). The spontaneous efflux of [^{3}H]HA (2 mM K^+) represented 14 ± 2% of the total [^{3}H]HA in synaptosomes. In the presence of added agents, [^{3}H]HA release induced by 30 mM K^+ (expressed in percent of total [^{3}H]HA over spontaneous efflux) represented 17 ± 2%. Each point represents the mean ± S.E.M. of results from three different experiments with quadruplicate determinations each.

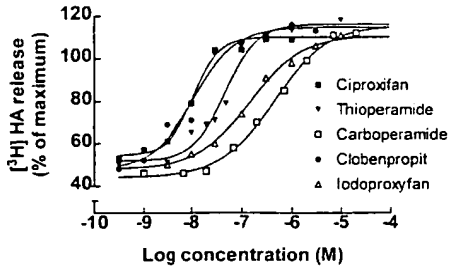

Fig. 3. Effects of H_3-receptor antagonists on the inhibition by HA of the K^+-induced [^{3}H]HA release from rat cerebral cortex synaptosomes. Synaptosomes preincubated with 1 μM HA alone or together with one of the compounds at increasing concentrations. They were depolarized for 2 min in the presence of 30 mM K^+. Each point represents the mean result from three different experiments with quadruplicate determinations each.

TABLE 1

In vitro and *in vivo* potencies of H_3-receptor antagonists

Compounds	*In Vitro* Tests (K_i, nM)		*In Vivo* Test (ED_{50}, mg/kg, p.o.)
	[^{3}H]HA release[a]	[^{125}I]Iodoproxyfan binding[b]	Increase in t-MeHA level in mouse brain[c]
Ciproxifan	0.5 ± 0.1	0.7 ± 0.2	0.14 ± 0.03
Thioperamide	4 ± 1	4 ± 1	1.0 ± 0.5
Carboperamide	20 ± 2	6 ± 2	3.9 ± 0.8
Clobenpropit	0.6 ± 0.2	0.5 ± 0.1	~25
Iodoproxyfan	5 ± 1	0.2 ± 0.1	>25

[a] Data from Fig. 3 except for thioperamide (from Arrang *et al.*, 1987) and iodoproxyfan (from Ligneau *et al.*, 1994).
[b] Data from Ligneau *et al.* (1994) except for ciproxifan (fig. 4).
[c] Data from figure 8 except for thioperamide (from Ganellin *et al.*, 1996) and iodoproxyfan (from Stark *et al.*, 1996).

n = 6), 105 ± 9 (100 nM, n = 6) and 87 ± 7% (300 nM, n = 6) *vs.* 100% relaxation in the absence of ciproxifan]. Schild analysis revealed a slope of 0.92 ± 0.04, not significantly different from unity (n = 26). After imposing the unity constraint, a pA_2 value of 8.38 ± 0.03 was calculated for ciproxifan.

Effect of ciproxifan and other H_3-receptor antagonists on [^{125}I]iodoproxyfan binding. At 25 pM [^{125}I]iodoproxyfan the specific binding to rat striatal membranes represented 8.4 ± 0.4 fmol/mg of protein, *i.e.*, 46 ± 1% of the total, and was completely and monophasically displaced by all compounds (fig. 4). From these displacement curves IC_{50}

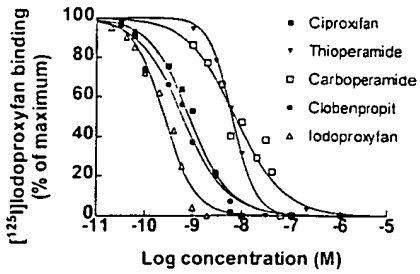

Fig. 4. Inhibition of [^{125}I]iodoproxyfan binding to rat striatal membranes by various histaminergic agents. Membranes were incubated for 60 min at 25°C with 25 pM [^{125}I]iodoproxyfan and unlabeled H_3-receptor antagonists in increasing concentrations. Specific binding, defined as that inhibited by 1 μM (*R*)α-MeHA, represented 46 ± 1% of the total binding. Results are expressed as percentages of specific [^{125}I]iodoproxyfan binding in the absence of unlabeled agents. Each point and vertical bars represent the mean ± S.E.M. of results from three different experiments with triplicate determinations each.

values for the compounds were deduced leading to the K_i values presented in table 1.

Receptor selectivity of ciproxifan. The compound displayed low apparent affinity at other receptor subtypes as evaluated in functional tests on isolated organs (histamine H_1 and H_2, muscarinic M_3, adrenergic α_{1D} and β_1, serotonin 5-HT_{1B}, 5-HT_{2A}, 5-HT_3 and 5-HT_4) (fig. 5). In addition, ciproxifan displayed a K_i value higher than 1 μM in a large variety of radioligand binding tests (Panlabs screen), except at [^{3}H]pirenzepine binding to rat cerebral cortex membranes where its K_i was about 1 μM (data not shown).

Changes in serum drug concentration in animals treated with ciproxifan. After i.v. administration of 1 mg/kg ciproxifan to mice (fig. 6B), the H_3-receptor ligand concentration in serum decreased progressively, fitting a typical biexponential decay model with half-times ($t_{1/2}$) of 13 and 87 min for the distribution and elimination phases, respectively. The quality of this fit is given by an R^2 value of 0.985. At 6 hr, the serum ligand concentration was still detectable with a value of 23 ± 6 nM. When ciproxifan was given orally, also at 1 mg/kg (fig. 5A), serum ligand level rose rapidly, being maximal at 30 min with a maximal concentration (C_{max}) value of 420 ± 40 nM; then, the ligand concentration

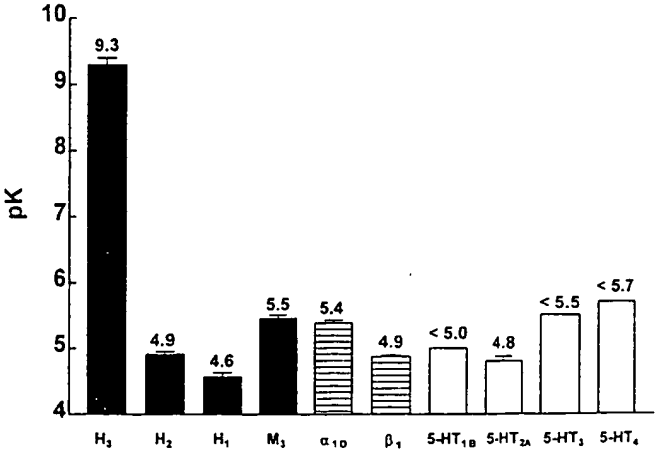

Fig. 5. Receptor selectivity profile of ciproxifan. The affinity of the compound at the H_3 receptor (pK_i from [^{3}H]HA release assay) is compared to corresponding values obtained in functional tests in isolated organs. Other values represent mean ± S.E.M. pA_2 (Arunlakshana and Schild, 1959) from n = 3 to 12 preparations except for the H_2- and β_1-receptor assay (pD'_2 according to Van Rossum, 1963).

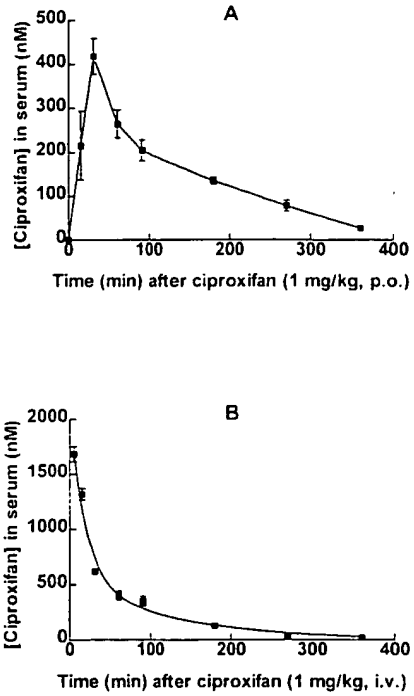

Fig. 6. Serum drug concentration in mice receiving ciproxifan. Mice were killed after p.o. (A) or i.v. (B) administration of ciproxifan (1 mg/kg), and drug concentrations were evaluated in serum by a radioreceptor assay. Means ± S.E.M. of values from six mice.

decreased but still remained measurable at 6 hr (27 ± 5 nM). The AUCs were 1425 and 890 nM.hr after i.v. and p.o. administrations, respectively, leading to an oral bioavailability coefficient (AUCp.o./AUCi.v..100%) of 62%.

In rats (n = 3) receiving 1 mg/kg ciproxifan p.o., the time-course of changes in serum ligand concentration were comparable to those in mice with a mean AUC of 2,225 nM.h, a C_{max} of 881 ± 322 nM, also observed at 30 min, and a level of 96 ± 4 nM at 6 hr (not shown).

In one cat receiving 3 mg/kg of ciproxifan orally serum levels (μM) were of 0.14 (0.5 hr), 4.0 (1 hr), 2.2 (1.5 hr), 0.27 (3 hr) and 0.12 (6 hr) leading to an AUC of 5.07 μM.hr at this dose.

Changes in brain t-MeHA level after administration of ciproxifan or other H_3-receptor antagonists. After the administration of ciproxifan (1 mg/kg, p.o.), brain t-MeHA level rose rapidly, being already significantly increased after 30 min, reaching a plateau between 90 and 180 min and still remaining enhanced after 270 min (fig. 7). In mice receiving pargyline, a monoamine oxidase inhibitor, t-MeHA level increased linearly with time at a rate of 55 ng/g/hr, whereas coadministration of pargyline and ciproxifan enhanced this rate to 120 ng/g/hr.

The dose-response curves of ciproxifan and a series of other H_3-receptor antagonists were established by measuring t-MeHA levels 90 min after oral administration (fig. 8). Ciproxifan, thioperamide and carboperamide, maximally increased t-MeHA level to an equivalent extents about 2-fold over basal values. However, clobenpropit, at the highest dosage tested (30 mg/kg, p.o.) maximally enhanced t-MeHA level by 45%, and iodoproxyfan (10 mg/kg, p.o.) did not significantly modify this level. The ED_{50} values of the compounds, derived from data of figure 8, are reported in table 1.

Similar experiments performed in rats receiving ciproxifan orally led to ED_{50} values (mg/kg) of 0.23 ± 0.04 in cerebral cortex, 0.28 ± 0.08 in striatum and 0.30 ± 0.08 in hypothalamus with similar maximal enhancements of about 100% (not shown).

The time-course of the changes in t-MeHA levels in mouse brain elicited by oral administration of 0.3 mg/kg ciproxifan and 3 mg/kg thioperamide (fig. 9) were analyzed in terms of AUCs, leading to values (in percent increase.hr) of 597 and 425, respectively.

Effect of ciproxifan on the water consumption induced by an H_3-receptor agonist. The H_3-receptor agonist (R)α-MeHA (10 mg/kg, i.p.) markedly enhanced water consumption in rats, an effect that was progressively reversed by coadministration of ciproxifan in increasing dosage, the ID_{50} of the antagonist being 0.09 ± 0.04 mg/kg (fig. 10). Ciproxifan alone (3 mg/kg) did not significantly modify water consumption (fig. 10).

Effect of ciproxifan in the five-choice task in rats. Analysis of variance revealed a significant drug × stimulus duration interaction [$F(1,9) = 12.19$, $P < .01$]. As shown in figure 11, reducing the duration of the visual stimulus to 0.25 sec resulted in a significant reduction in the accuracy of performance compared to the baseline (0.5 sec) stimulus condition. Newman Keuls *post hoc* comparisons revealed that this reduction in performance was significant and that choice accuracy significantly increased after administration of 3.0 mg/kg of ciproxifan under the shorter stimulus condition compared to performance after administration of the vehicle ($P < .05$). There was no significant effect of this manipulation of the stimulus duration or of ciproxifan on any of the other measures recorded (*i.e.*, speed, anticipatory or perseverative responses and errors of omission).

Effects of ciproxifan on neocortical EEG power spectral density and sleep-wake cycle in cat. Administration

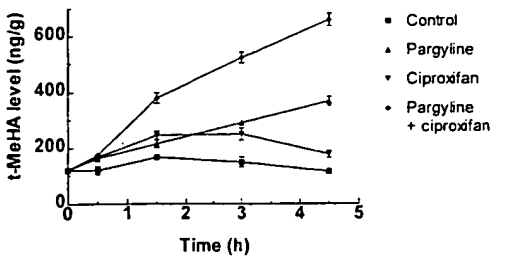

Fig. 7. Changes in brain t-MeHA levels in control and pargyline-treated mice after administration of ciproxifan. Mice were killed at various times after simultaneous administration of vehicle or ciproxifan (1 mg/kg, p.o.) and vehicle or pargyline (65 mg/kg, i.p.). t-MeHA levels are expressed in ng/g of tissue. Means ± S.E.M. of values from 12 mice.

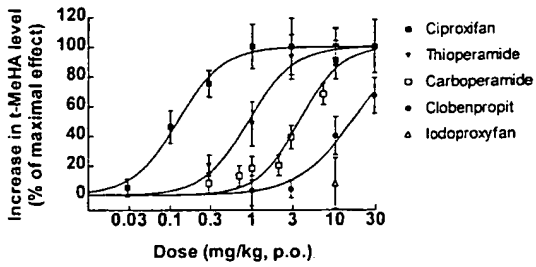

Fig. 8. Changes in brain t-MeHA levels in mice receiving H_3-receptor antagonists. Mice were killed 90 min after the p.o. administration of vehicle or drugs in increasing doses. t-MeHA levels in treated mice are expressed in percent increase as compared to levels in control mice (133 ± 7 ng/g). Means ± S.E.M. of values from 12 mice.

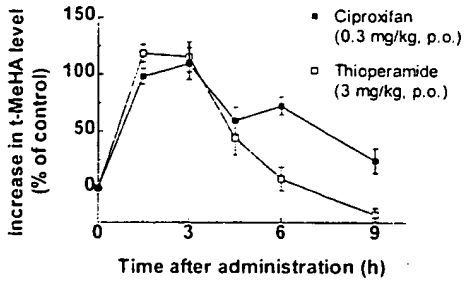

Fig. 9. Changes in brain t-MeHA levels in mice receiving ciproxifan or thioperamide. Mice were killed at various times after the p.o. administration of vehicle, ciproxifan (0.3 mg/kg) or thioperamide (3 mg/kg). t-MeHA levels are expressed in percent increase as compared to levels in corresponding controls. Means ± S.E.M. of values from 18 mice.

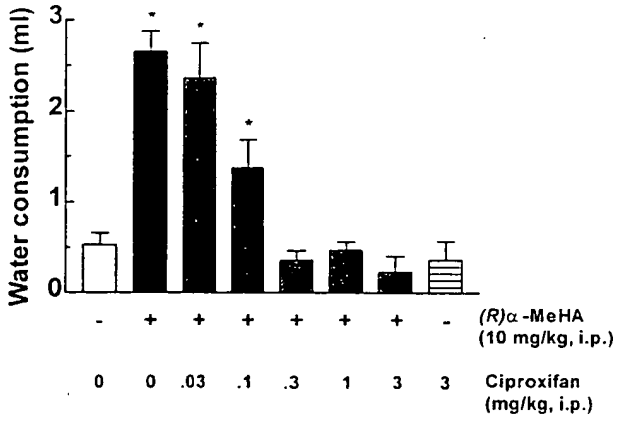

Fig. 10. Effect of ciproxifan on the (*R*)α-MeHA-induced water consumption in rats. Water consumption was measured over a 10-min period, 30 to 40 min after i.p. administrations of (*R*)α-MeHA and ciproxifan. Means ± S.E.M. of values from 5 to 10 rats per treatment group (data from two experiments). *P < .05 *vs.* vehicle.

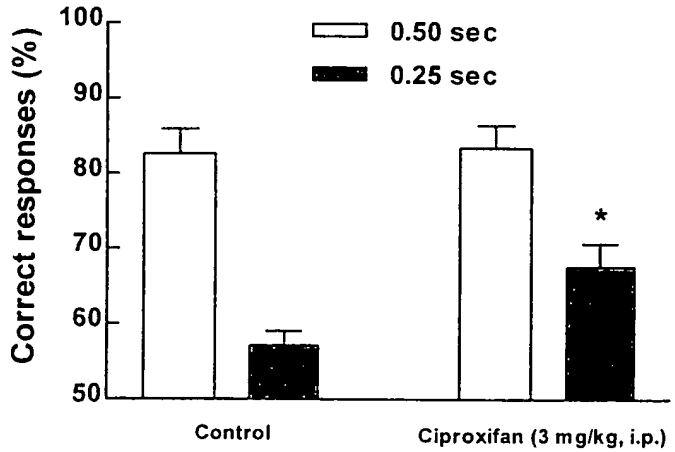

Fig. 11. Effects of ciproxifan in the five-choice task in rats. The drug was administered (3 mg/kg, i.p.) 1 hr before the test session. The accuracy of responding, expressed as the percentage of correct responses, was significantly (*P < .05) improved by treatment only when the stimulus duration was 0.25 sec instead of 0.50 sec.

of ciproxifan caused suppression or diminution (depending on the dose used) of neocortical slow activity (0.8–5 Hz) and spindles (8–15 Hz), resulting in a total cortical activation, *i.e.*, low voltage electrical activity with dominant waves in the β and γ bands (mainly 25–45 Hz). Furthermore, ciproxifan increased the power density of these neocortical fast rhythms (fig. 12). These effects, occurring within 25 min after

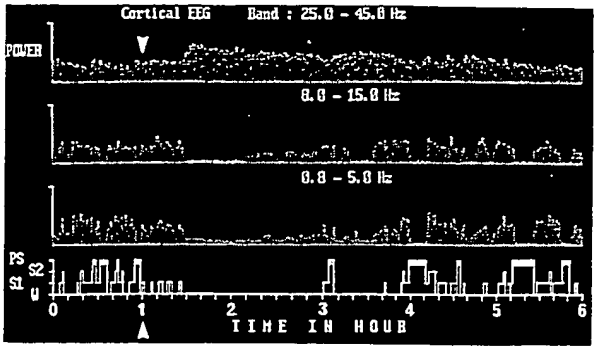

Fig. 12. An example of the effects of oral administration of ciproxifan on cortical EEG and sleep-wake cycle in cat. Neocortical EEG power density (mV^2) in different frequency bands and sleep-wake cycle 1 hr before and up to 5 hr after administration of 2 mg/kg ciproxifan (arrow) are given. Note, from bottom to top, that ciproxifan elicited a total waking state accompanied by a suppression of cortical slow frequency activity (0.8–5 Hz) and spindle (8–15 Hz) and a marked increase in fast rhythms (25–45 Hz). Abscissa, Time in hours; ordinate, sleep-wake stages (PS, paradoxical sleep; S2, deep slow wave sleep; S1, light slow wave sleep; W, wakefulness).

administration (fig. 12) were detectable at a dose of 0.15 mg/kg and became evident at a dose of 0.3 mg/kg or more (fig. 13).

The effects of ciproxifan on neocortical EEG were manifested on polygraphic scoring as an almost total waking state, the duration of which was dose dependent. This waking effect was characterized by an increase in wake-episode duration and a delayed sleep latency. During the same period both slow wave sleep (especially S2) and PS were suppressed (fig. 13). After the period of induced total waking, cortical slow activity gradually reappeared, but an increase in waking could be seen during a period proportional to the doses used. No obvious sleep rebound was noted after the waking effect, and all sleep-wake parameters including power spectral density of neocortical EEG returned to control levels on the next day. The arousal effects of ciproxifan (0.3 mg/kg) were prevented or significantly reduced by prior (15 min) systemic injection of either mepyramine (1 mg/kg), an H_1-receptor antagonist, or imetit (3 mg/kg), an H_3-receptor agonist (not shown).

Discussion

From our present *in vitro* and *in vivo* studies, ciproxifan appears as a pure competitive antagonist at the histamine H_3 receptor, one of the most potent so far available.

Our selection of the molecule was initially based on its ability to block the actions of histamine or imetit, a selective H_3-receptor agonist, at the autoreceptor regulating the release of neosynthesized [^{3}H]HA from K^+-depolarized synaptosomes, according to a previously described model (Arrang *et al.*, 1983; Garbarg *et al.*, 1989, 1992). On this model, ciproxifan induced a parallel rightward shift of the concentration-response curve to imetit, indicative of a competitive antagonism with an apparent dissociation constant in the low nanomolar range. Ciproxifan in increasing concentration also progressively blocked the (*R*)α-MeHA-induced relaxation of the electrically stimulated longitudinal muscle of guinea pig ileum in a competitive fashion. At these various functional models as well as the [^{125}I]iodoproxyfan binding tests, the drug displayed similarly low apparent dissociation constants

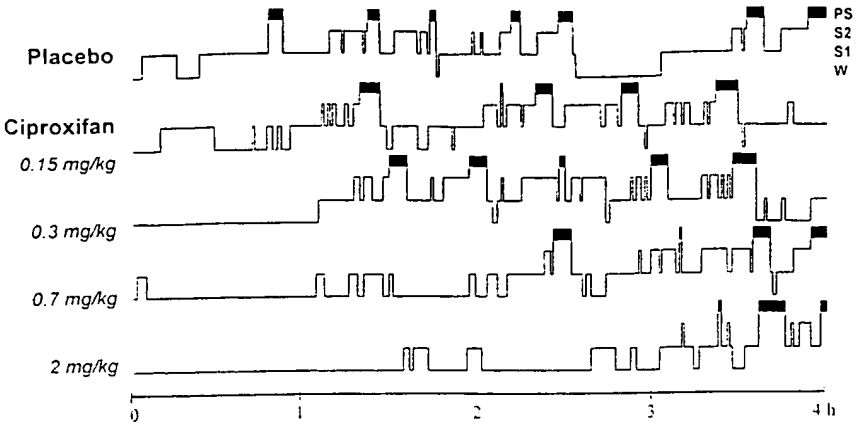

Fig. 13. Representative hypnograms (4 hr) obtained in cats after oral administration of ciproxifan at different doses. Note the dose-dependent waking effect. Abscissa, Time in hours; ordinate, sleep-wake stages (PS, paradoxical sleep; S2, deep slow wave sleep; S1, light slow wave sleep; W, wakefulness).

(K_i = 0.5–4.2 nM), an observation that does not support the hypothesis of the existence of H_3-receptor subtypes (Clapham and Kilpatrick, 1992). The hypothesis, mainly based on discrepancies in potency of some compounds in binding and functional models, is not supported either by the closely similar potencies of other antagonists in different models, examplified in table 1 (with the exception of iodoproxyfan for which the discrepancy can be fully explained by a slow equilibration rate).

The high degree of selectivity of ciproxifan toward the histamine H_3 receptor was shown by the observation that the drug displayed lower affinity by about three orders of magnitude for any other receptor subtype on which it was tested (see "Results").

Although some compounds *in vitro* were as potent as or even more potent than ciproxifan at the H_3 receptor, *e.g.*, clobenpropit and iodoproxyfan, ciproxifan given orally to mice enhanced brain t-MeHA levels at much lower dosage (ED_{50} = 0.14 mg/kg) than any of these compounds. A similar change, occurring with ED_{50} of 0.2–0.3 mg/kg p.o., was found in various areas of rat brain. t-MeHA is the product of the major metabolic pathway for endogenous HA in brain (Schwartz *et al.*, 1971), and its steady-state level is a reliable index of histaminergic neuron activity (Oishi *et al.*, 1983). The increase in t-MeHA level induced by the H_3-receptor antagonists corresponds to an enhanced HA release, reflecting the tonic inhibition that the endogenous amine exerts on this process and on neuronal firing via stimulation of autoreceptors in the somatodendritic or terminal area of histaminergic neurons. In agreement, ciproxifan (1 mg/kg, p.o.) enhanced HA turnover rate in mouse brain, evaluated from the rate of t-MeHA accumulation after monoamine oxidase inhibition, from a value of 55 ng/g/hr, consistent with corresponding values obtained in the same species using either isotopic (Verdière *et al.*, 1977) or nonisotopic methods (Oishi *et al.*, 1989), to a value of 120 ng/g/hr.

A similar maximal effect, corresponding to a nearly doubling of t-MeHA level, was obtained with thioperamide or carboperamide whereas, at the maximal dose tested of clobenpropit (30 mg/kg), this level was not reached and no significant change occurred after administration of iodoproxyfan (fig. 8) despite the high potency of these compounds *in vitro*.

In the case of ciproxifan, a rather high oral bioavailability was evidenced by the ratio (>60%) of AUCs of H_3 receptor binding activity in blood serum, measured by using a novel radioreceptor assay, following drug administration (1 mg/kg) by p.o. and i.v. routes, respectively. The rather slow kinetics of ciproxifan are indicated by a serum level still about 10 times above the K_i value of the drug at the H_3 receptor 6 hr after oral administration. At this time, t-MeHA levels in brain are still enhanced by 24 ± 11% (fig. 9). Such comparison between drug levels in blood and a typical brain response in rodents might be useful to predict effective dosages in other species, particularly humans, in which only blood levels are available, assuming a similar ability of the drug to cross the blood-brain barrier. A similarly favorable bioavailability of ciproxifan on oral administration to rats and cats is suggested by measurements of t-MeHA and drug serum levels, respectively (see "Results").

Characteristic behavioral responses were found in rats and cats receiving ciproxifan in low dosage. In water-deprived rats, ciproxifan blocked the enhancement of drinking elicited by (*R*)α-MeHA, an H_3-receptor agonist (Clapham and Kilpatrick, 1993), with an ID_{50} value of ~0.1 mg/kg. The exact site (central or peripheral) and mechanism of action of H_3-receptor ligands in this test has not been clearly established. Thus, whereas the involvement of the renin-angiotensin system in HA-induced drinking was postulated by Kraly and Miller (1982), an AT_1 antagonist did not affect the (*R*)α-MeHA-induced drinking (Clapham and Kilpatrick, 1993). The observation that (*R*)α-MeHA-induced drinking is blocked at doses of ciproxifan (this study), thioperamide and particularly clobenpropit (Barnes *et al.*, 1993), close to those enhancing endogenous HA release in brain (table 1), suggests that H_3 receptors in brain rather than in periphery are involved. The observation that ciproxifan or thioperamide given alone do not affect drinking suggests that the effect of (*R*)α-MeHA is mediated by H_3 hetero- rather than autoreceptors. In agreement, H_3 receptors on noradrenergic, serotoninergic, cholinergic, dopaminergic or peptidergic neurons do not appear to be, as with those on histaminergic neurons, tonically modulated by endogenous HA, because they respond to agonists but not to antagonists given alone (Schwartz *et al.*, 1991, 1995; Schlicker *et al.*, 1994).

The marked dose-dependent waking effect of ciproxifan in cats is consistent with a large variety of experimental evidence showing that histaminergic neurons play a prominent role in cortical activation and arousal in cats and rats (reviewed by Lin *et al.*, 1996; Schwartz *et al.*, 1991, 1995). The arousing effects of H_3-receptor antagonists, characterized by an enhancement of wakefulness at the expense of slow wave

and paradoxical sleep, was previously shown in both animal species using thioperamide (Lin *et al.*, 1990; Monti *et al.*, 1991) and carboperamide (Monti *et al.*, 1996). The effect of ciproxifan was, as in the case of thioperamide (Lin *et al.*, 1990), prevented by administration of mepyramine, an H_1-receptor antagonist, suggesting that it resulted from a H_3-receptor mediated enhancement of endogenous HA release. The brain site(s) at which endogenous HA promote(s) cortical EEG desynchronization via activation of the H_1 receptor could be one of the brain areas to which ascending or descending histaminergic pathways project known to express the H_1 receptor and to control sleep/wakefulness states. These potential targets comprise cortical neurons receiving direct histaminergic projections from the tuberomammillary nucleus, preoptic anterior hypothalamic neurons, thalamic relay neurons and basal forebrain or mesopontine tegmentum neurons (Lin *et al.*, 1996).

Because the effect of ciproxifan in cats was to enhance fast cortical rhythms, known to occur during increased vigilance, and to cause a quiet waking state, a positive outcome in attentional tests could be anticipated. In confirmation the drug significantly enhanced choice accuracy in the five-choice serial reaction-time task when a visual stimulus of short duration (0.25 sec) was used. Such reduction of the stimulus duration increases the attentional load placed on the task, reduces choice accuracy and has been used to observe the effects of cholinergic agents on attentional function (Muir *et al.*, 1994, 1995). "Pro-cognitive" effects of the H_3-receptor antagonist thioperamide have been reported in other behavioural tasks, *e.g.*, step-through passive avoidance response in senescence-accelerated mice (Meguro *et al.*, 1995); elevated plus-maze performance in mice with scopolamine-induced learning deficits (Miyazaki *et al.*, 1995) and in a test of social memory in rats (Prast *et al.*, 1996). However, it has also been reported that thioperamide failed to improve scopolamine-induced attentional dysfunction in the same 5-choice task used in the present study (Kirkby *et al.*, 1996).

Taken together these various observations suggest that ciproxifan is a potent, orally active H_3-receptor antagonist and it seems of interest to assess its potential therapeutic applications, namely in aging or degenerative disorders in which vigilance, attention and memory are impaired.

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PHYSIOLOGICAL REVIEW ARTICLE

Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons

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Wakefulness is a functional brain state that allows the performance of several "high brain functions", such as diverse behavioural, cognitive and emotional activities. Present knowledge at the whole animal or cellular level suggests that the maintenance of the cerebral cortex in this highly complex state necessitates the convergent and divergent activity of an ascending network within a large reticular zone, extending from the medulla to the forebrain and involving four major subcortical structures (the thalamus, basal forebrain, posterior hypothalamus and brainstem monoaminergic nuclei), their integral interconnections and several neurotransmitters, such as glutamate, acetylcholine, histamine and noradrenaline. In this mini-review, the importance of the thalamus, basal forebrain and brainstem monoaminergic neurons in wake control is briefly summarized, before turning our attention to the posterior hypothalamus and histaminergic neurons, which have been far less studied. Classical and recent experimental data are summarized, supporting the hypothesis that (1) the posterior hypothalamus constitutes one of the brain ascending activating systems and plays an important role in waking; (2) this function is mediated, in part, by histaminergic neurons, which constitute one of the excitatory sources for cortical activation during waking; (3) the mechanisms of histaminergic arousal involve both the ascending and descending projections of histaminergic neurons and their interactions with diverse neuronal populations, such as neurons in the pre-optic area and cholinergic neurons; and (4) other widespread-projecting neurons in the posterior hypothalamus also contribute to the tonic cortical activation during wakefulness and/or paradoxical sleep.

Key words: wakefulness, cortical activation, posterior hypothalamus, histaminergic neurons, basal forebrain, thalamus, mesopontine tegmentum, cholinergic neurons, noradrenergic neurons, locus coeruleus.

Introduction

As a result of classical investigations using brain transection, lesioning, or electric stimulation, it is generally believed that the cerebral cortex does not possess an intrinsic

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[3–6, 9, 10]. Although the reticulothalamocortical pathway is still regarded as one of the most important substrates for ascending cortical activation, it cannot be considered as the only system involved, as cortical EEG desynchronization can reappear following extensive cellular destruction of the mesencephalic reticular formation [11] or its thalamic relay [12–15]. It is therefore reasonable to imagine the existence of one, or several, extrathalamic systems, capable of activating the cortex, especially in the case of a deficit of the reticulothalamocortical system. Among such systems, the magnocellular substantia innominata and adjacent basal forebrain and, in particular, the cholinergic and gabaergic corticopetal neurons have received special attention (see Refs 5–7 for reviews).

The basal forebrain in cortical activation

As a result of their studies using acetylcholinesterase as a marker, Shute and Lewis proposed, as early as the 1960s [16], that the dense acetylcholinesterase-stained pathways from the brainstem to the subcortical regions and those from the basal forebrain to the cortex might serve as a neuronal substratum for the concept of an ascending reticular activating system. Nowadays, we know that this system corresponds roughly to the ascending cholinergic diencephalic projections from the mesopontine tegmentum, as well as those from the basal forebrain to the neocortex [17]. Basal forebrain presumed cholinergic neurons discharge tonically during both wakefulness and PS and have been suggested [5, 15] to play an important role, either directly (by post-synaptic excitation) in activating cortical cells or indirectly in suppressing the neuronal rhythmic oscillation of the thalamic reticular nucleus, responsible for the generation of cortical spindles, indicative of drowsiness or light SWS [4]. In support of this, electrical stimulation of certain basal forebrain sites elicits cortical acetylcholine release accompanied by cortical desynchronization, while chemical inactivation or unilateral lesion in the basal forebrain cholinergic zone can decrease cortical fast rhythms and increase slow activity [15, 18, 19]. More recent *in vivo* and *in vitro* studies have suggested that, like thalamocortical neurons, basal forebrain cholinergic neurons are able to relay excitatory influxes (such as those from glutamatergic, noradrenergic and histaminergic neurons) from the lower brain reticular structures to the cortex [7, 20]. The gabaergic ascending neurons that are codistributed with cholinergic neurons in the basal forebrain also project to the cortex [7] and might act in synergy with the cholinergic neurons in cortical activation, probably by ascending disinhibitory mechanisms, because they principally innervate cortical inhibiting neuronal elements [21]. Thus, there is little doubt that the substantia innominata and the adjacent basal forebrain as a whole, including cholinergic, gabaergic, and perhaps non-identified neurons, play an important role in cortical activation both during waking and PS and in the modulation of different cortical rhythmic activities. However, it seems unlikely that this role is exclusive and that the basal forebrain is indispensable for the long-term maintenance of cortical low voltage and fast activity, since, in the cat, extensive destruction of the basal forebrain, including the adjacent lateral pre-optic areas, does not abolish cortical activation [22], while ibotenic acid lesioning, aimed at destroying the cholinergic zone within the basal forebrain, elicits a transitory reduction in waking lasting 1–2 days, after which the sleep–wake cycle returns to the pre-lesioning level ([23] and our unpublished data).

lateral limit is the pes pedunculi and subthalamic nucleus. Caudally, the posterior hypothalamus is in continuity with the ventral tegmental area of Tsai and, rostrally, the ventromedial hypothalamic nucleus roughly separates it from the pre-optic/anterior hypothalamus, a brain region known for its importance in sleep generation. The posterior hypothalamus consists of a large reticular zone containing neurons of small to middle size (with the exception of the peri-, supra- and tubero-mammillary regions in which distinct nuclei made up of relative large cells are present) and ascending and descending fibers of passage. The heterogeneity of this region is also reflected by the presence of many neurotransmitters or neuroactive substances, for example:

(1) gabaergic and most probably glutamatergic neurons [36], and neurons containing peptides (such as enkephalin, substance P, galanine, or thyrotropin-releasing hormone), all these neurons being distributed in a quite diffuse manner;
(2) the dopaminergic A11 group, mainly situated in the lateral and dorsal areas and periventricular substance gray, and projecting to the large hypothalamic areas and the mesopontine tegmentum, including the locus coeruleus [37];
(3) in the dorsolateral area, neurons containing melanin-concentrating hormone (MCH) [38, 39], or the neuropeptide, hypocretin/orexin, situated mainly in the perifornical region and adjacent zone in rats [40, 41]; it seems likely that prolactin-like substance is also present in neurons containing orexin [42]. All these neurons have widespread projections in the brain;
(4) in the ventrolateral region, histaminergic cell bodies in the tuberomammillary (TM) nucleus and adjacent areas ([43–47], see later section). In the rodent, various percentages of these neurons are reported to contain GABA, adenosine deaminase, or, after colchicine treatment, certain peptides, such as galanine or substance P [48, 49]. These coexisting substances are also present elsewhere in the brain, with only the presence of histamine being anatomically exclusive to these TM neurons.

The posterior hypothalamus receives both large descending inputs from the pre-optic/anterior hypothalamus and other forebrain structures (such as gabaergic and peptidergic afferents) and ascending inputs arising from the brainstem (such as cholinergic and gabaergic afferents from the mesopontine tegmentum and monoaminergic afferents from the brainstem [see details in [50–54]).

A region classically referred as a "waking center"

Unlike other brain structures involved in the control of cortical activation and waking, which have been intensively studied during the last decades [4–7, 27], the posterior hypothalamus has not received much attention. However, the history of its functional importance in waking can be traced back as far as 1926 when von Economo initially observed hypersomnia in patients suffering from inflammatory lesions within this region [55]. Subsequently, many studies conducted in experimental animals (cats, monkeys and rats) have confirmed this observation by demonstrating that somnolence and hypersomnia can be caused by electrolytic lesioning of the posterior hypothalamus (see Ref. 3 for review). Thus, as early as 1946, Nauta, on the basis of lesion studies in the rat hypothalamus, defined a waking center located in the posterior hypothalamus and a sleep center located in the pre-optic/anterior hypothalamus. He further suggested

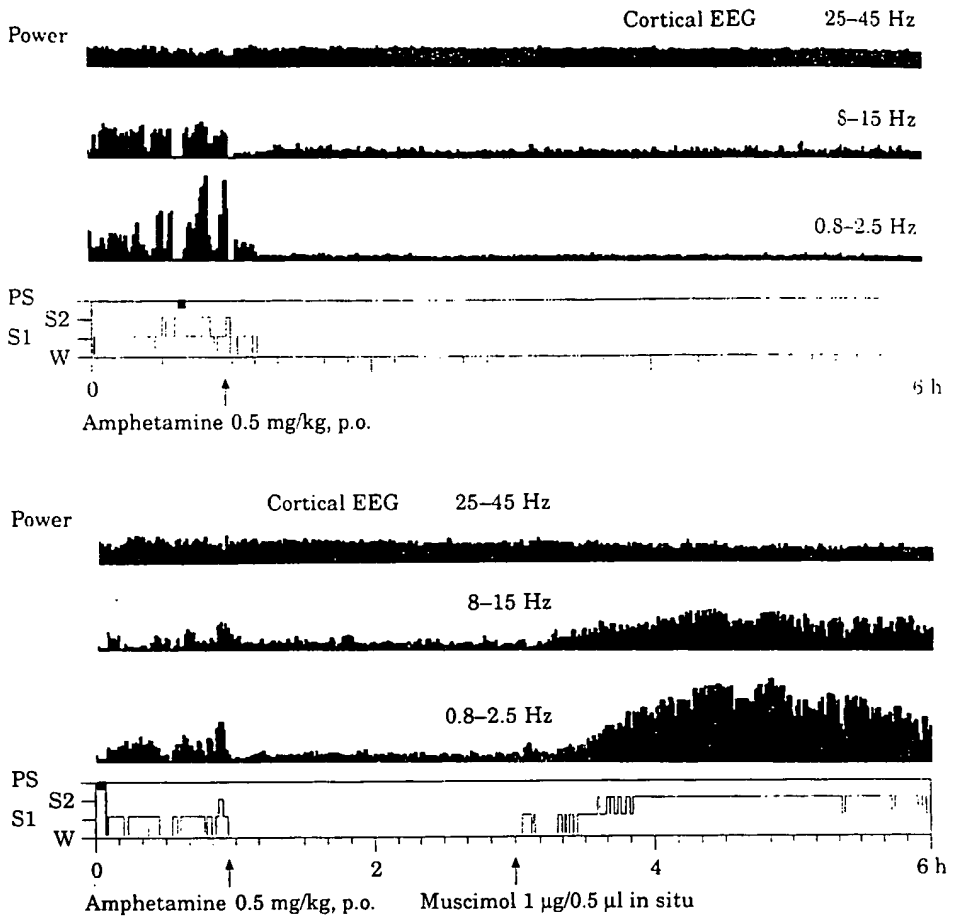

Figure 1 Insomnia in the cat induced by oral application of amphetamine and its reversibility by subsequent micro-injection of muscimol into the tuberomammillary area. Representative 6-h hypnograms and cortical power spectra at 0.8–2.5, 8–15 and 25–45 Hz are shown. Note that amphetamine (0.5 mg/kg) causes a long-lasting waking state accompanied by suppression of cortical spindles (8–15 Hz) and slow activity (0.8–2.5 Hz) (upper panels) and that muscimol (1 µg/0.5 µl) induces reappearance of slow wave sleep with both cortical slow activity and spindles with short latency (lower panels). X-axis: time in hours; Y-axis: sleep–wake stages (PS, paradoxical sleep; S2, deep slow wave sleep; S1, light slow wave sleep; W, wakefulness) ($n = 3$, from author's unpublished data).

posterior hypothalamus induces pronounced and long-lasting increase in deep SWS, accompanied by a reduction in, or suppression of, PS.

(3) When the injection is performed in the caudal part, the increase in deep SWS is followed by an increase either in waking or PS, depending upon the exact injection site. In the latter case, PS can even directly follow waking.

(4) In both the rostral and caudal parts, induction of deep SWS is seen with a shorter latency when injection is made ventrally, rather than dorsally [64].

We have, therefore, defined the rostral and middle parts of the posterior hypothalamus as the main hypothalamic waking territory (see Fig. 9B), which is, up to

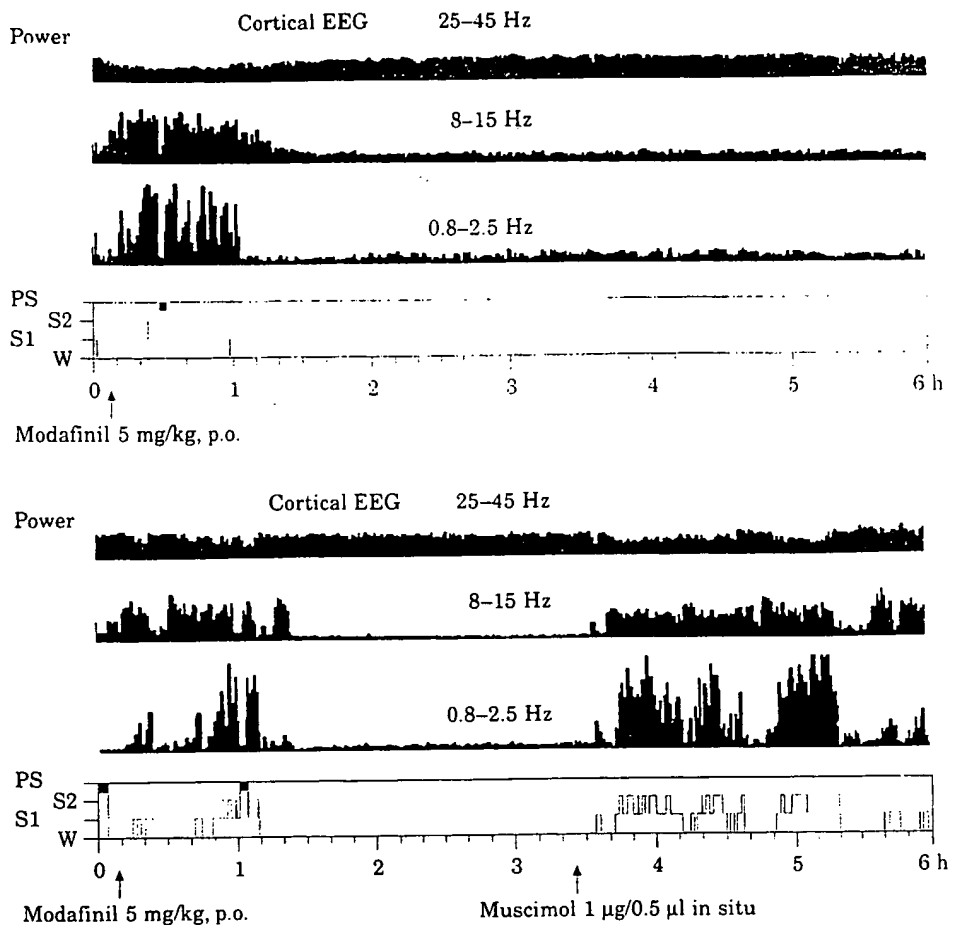

Figure 2 Long-lasting wakefulness in the cat induced by oral application of modafinil and its reversibility by subsequent micro-injection of muscimol into the tuberomammillary area. Representative 6-h hypnograms and cortical power spectra at 0.8–2.5, 8–15 and 25–45 Hz are shown. Note that modafinil (5 mg/kg) causes a waking state with a latency of about 1 h (top panels) and that muscimol (1 µg/0.5 µl) induces reappearance of slow wave sleep with both cortical slow activity and spindles with short latency (bottom panels). For abbreviations, see legend to Fig. 1 ($n=3$, from author's unpublished data).

blocked by muscimol micro-injection into the TM area, inducing the reappearance of SWS with short latency, accompanied by both cortical slow activity and spindles (Fig. 2, lower panels).

From these results, it therefore seems clear that inactivation of the posterior hypothalamus induces hypersomnia in normal cats and restores sleep in various models of insomnia provoked by different experimental means, suggesting the key role of this region in the mechanisms and maintenance of cortical activation and waking state. The data also suggest that the posterior hypothalamus should also be crucially involved in mechanisms of insomnia and its neuronal populations should be in a state of hyperactivation in the different models of insomnia, such that neuronal inactivation

neuromodulator [75–77] in the brain was it possible to hypothesize that a blockade of histaminergic transmission could be responsible for the drowsiness caused by antihistaminics.

Histaminergic cells as one of the neuronal components of the posterior hypothalamus involved in cortical activation of waking

Histaminergic neurons and their fiber projections

In the mammalian brain, histamine is synthesized from L-histidine by histidine decarboxylase and is predominantly inactivated by histamine-N-methyltransferase. The central actions of histamine are mediated by post-synaptic H1- and H2-receptors and by H3-receptors possessing characteristics of auto- and hetero-receptors [75–77]. In addition to a large body of neuroanatomical data obtained in rats and other species by other groups [e.g. 43–45, 77], we have demonstrated, in the cat [23, 46, 47, 78], that histaminergic neurons are located exclusively in the TM nucleus and adjacent areas of the posterior hypothalamus (Fig. 3, lower part), a brain area of which the importance in waking has been summarized above. As schematically summarized in Figure 3 (upper part), these neurons send widespread ascending and descending inputs to various brain areas, especially those known for their importance in controlling the sleep–wake states, such as the cortex, thalamus, pre-optic/anterior hypothalamus, periaqueducal gray, and adjacent areas, and to brainstem and forebrain cholinergic and monoaminergic neurons. In addition, histamine is also present in mast cells and the endothelium of micro-vessels, whereas histamine H1- and H2-receptors have been identified not only on neurons, but also on glial cells [77], suggesting that non-neuronal histamine may also regulate brain functions.

Consistent with the widespread inputs of histaminergic neurons, we have recently used *in situ* hybridization [79, 80] to demonstrate strong expression of histamine H1-receptor mRNA in neuronal populations of large brain areas in the guinea-pig, e.g. in the cortex, intralaminar nuclei of the thalamus and mesencephalic reticular formation (see also Refs 81, 82). H2-receptor mRNA also has been identified in large cell populations in the cortex, thalamus and basal forebrain in guinea-pigs [83].

Discharge pattern of histaminergic neurons and post-synaptic actions of histamine

The anatomical organization of histaminergic neurons suggests that this system might influence the neuronal excitability and activity of large brain areas. Electrophysiological data support this idea. In the rat brain slice *in vitro*, histaminergic neurons are spontaneously active with a discharge rate of 2.1 ± 0.6 Hz [84, 85]. In the freely-moving cat, presumed histaminergic neurons present a waking state-specific activity, discharging tonically and specifically during wakefulness, with their activity decreasing significantly as soon as cortical spindles appear (drowsiness) and becoming totally silent during deep SWS and PS. In the cat hypothalamus, this kind of activity is exclusively recorded in the TM and adjacent ventrolateral posterior hypothalamus [52, 60]. Similar state-dependent discharge patterns have been recently recorded in the TM and adjacent areas in freely-moving rats [62].

Consistent with the high discharge rate of presumed histaminergic neurons during waking, it has been demonstrated in rodents that the release and turnover of histamine is high during darkness, the period in which the animals are active and spend a large part of their time in waking [86, 87]. In monkeys, increased histamine release in the posterior hypothalamus occurs on awakening and is maintained during each wake episode [88].

At the post-synaptic level, recent *in vitro* intracellular recording studies have revealed that activation of H1-receptors on thalamic relay neurons results in slow depolarization by suppression of leakage potassium current. Activation of H2-receptors, on the other hand, induces a small depolarization, associated with an increase in hyperpolarization current. As a result, this combined action of histamine can cause a switch of neuronal discharge from rhythmic bursts to tonic activity, and therefore might promote the switch from SWS to waking [10]. Similar results have been obtained in human cortical neurons [89] and guinea-pig mesopontine and basal forebrain cholinergic neurons [20, 90]. Activation of H2-receptors also causes shortening of afterhyperpolarization and may facilitate cortical and hippocampal activity [84, 85]. Moreover, in rat cortical or hippocampal cultures or isolated neurons, histamine has been shown to enhance NMDA receptor-mediated synaptic transmission by as yet undefined receptors and mechanisms [91, 92].

These electrophysiological data suggest that histaminergic cells increase their activity during wake state and activate or facilitate the neuronal activity of large brain areas, such as the cortex, thalamus, and basal forebrain, thus contributing to generalized cortical activation. In order to evaluate this assumption, we have carried out a series of systemic and *in situ* pharmacological studies in the cat; these data are summarized below.

Histaminergic transmission and the sleep–wake cycle

Firstly, in cats [93] as in rats [94, 95], intraperitoneal injection of α-fluoromethylhistidine, a specific inhibitor of the histamine-synthesis enzyme, induces a slowly-developed and significant decrease in waking and an increase in SWS without modifying PS, the time course of the effects paralleling the reduction in brain histamine content. Similarly, intraperitoneal injection of mepyramine, a H1-receptor antagonist, causes an increase in cortical slow waves, as revealed by power spectral analysis, this slow activity being indistinguishable from that seen during SWS in the control recording (Fig. 4, upper part). This effect is seen on EEG scoring as a decrease in latency to SWS and an increase in the time spent in SWS, at the expense of a decrease in both waking and PS (see Fig. 4, lower part and Refs 23, 92). In man, H1-receptor antagonists have been shown to impair vigilance during waking and to reduce performance [96]. These data obtained using H1-receptor antagonists, are consistent with the sedation and drowsiness caused in man by classical antihistaminics [74], now designated as H1-receptor antagonists [77] and provide experimental confirmation to this well-known clinical observation.

Secondly, intraventricular injection of ranitidine, a H2-receptor antagonist, also increases SWS [23] in the cat, the effect being slow and progressive as shown in Figure 5. Whereas the exact role of H2-receptors on sleep–wake cycle remains to be established by using potent and specific brain-penetrating agonists and antagonists, the effect seen

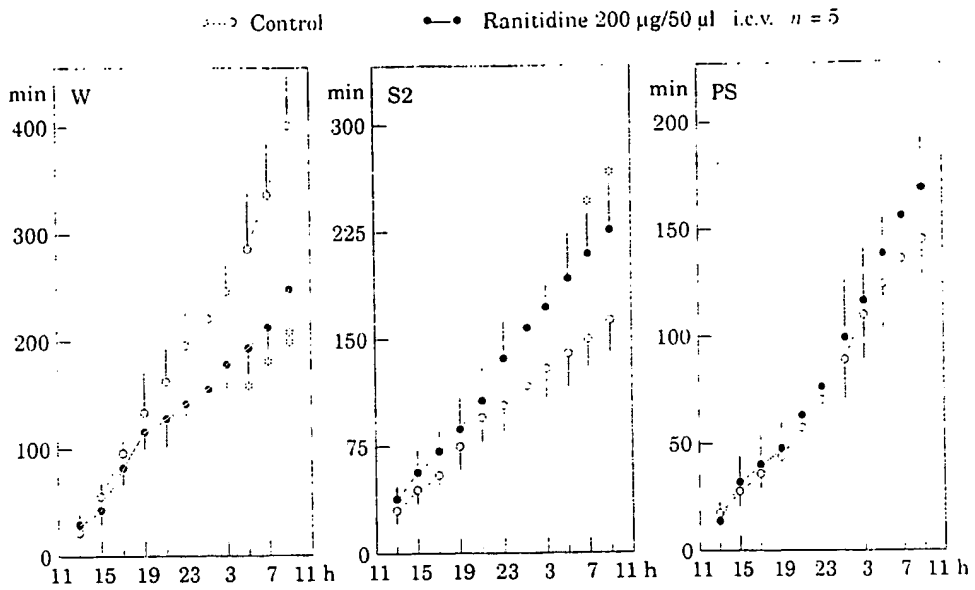

Figure 5 Effects of ranitidine (a H2-receptor antagonist) on the sleep–wake cycle in the cat. The curves show the mean 2-h cumulative values ± SEM (vertical bars) for wakefulness (W), light slow wave sleep (S1), deep slow wave sleep (S2), and paradoxical sleep (PS) during the 22 h (11.00–09.00 hours next day) following intraventricular injection of Ringer's solution (50 µl) or ranitidine (200 µg/50 µl) at 11.00 hours. See text for other comments. X-axis: amount of sleep–wake states in min. Y-axis: local time at 2-h period (* $P<0.01$; ** $P<0.02$; Student's t-test) (based on the author's work in Ref. 23).

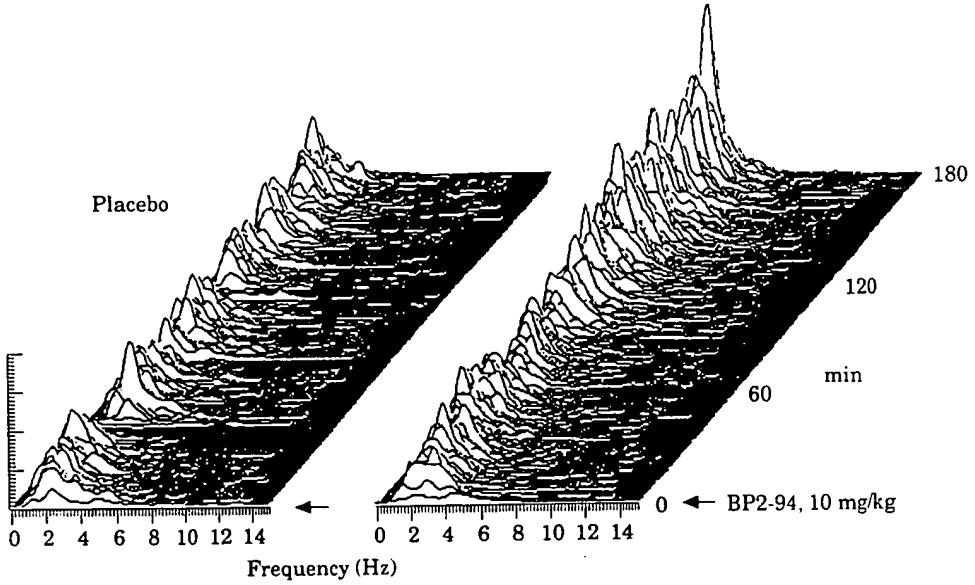

Figure 6 A representative example of the effect of BP2-94 (a H3-receptor agonist) on the neocortical EEG power spectral density in the cat. The EEG spectra (up to 15 Hz) were obtained by fast Fourier transformation within 3 h of oral administration of placebo or BP2-94 (10 mg/kg). Note the increase in power spectral density of slow activity (up to 5 Hz range). 0 corresponds to the time of administration. X-axis, EEG frequency in Hz; Y-axis, EEG power; Z-axis, time in min ($n=5$; based on the author's work in Ref. 23).

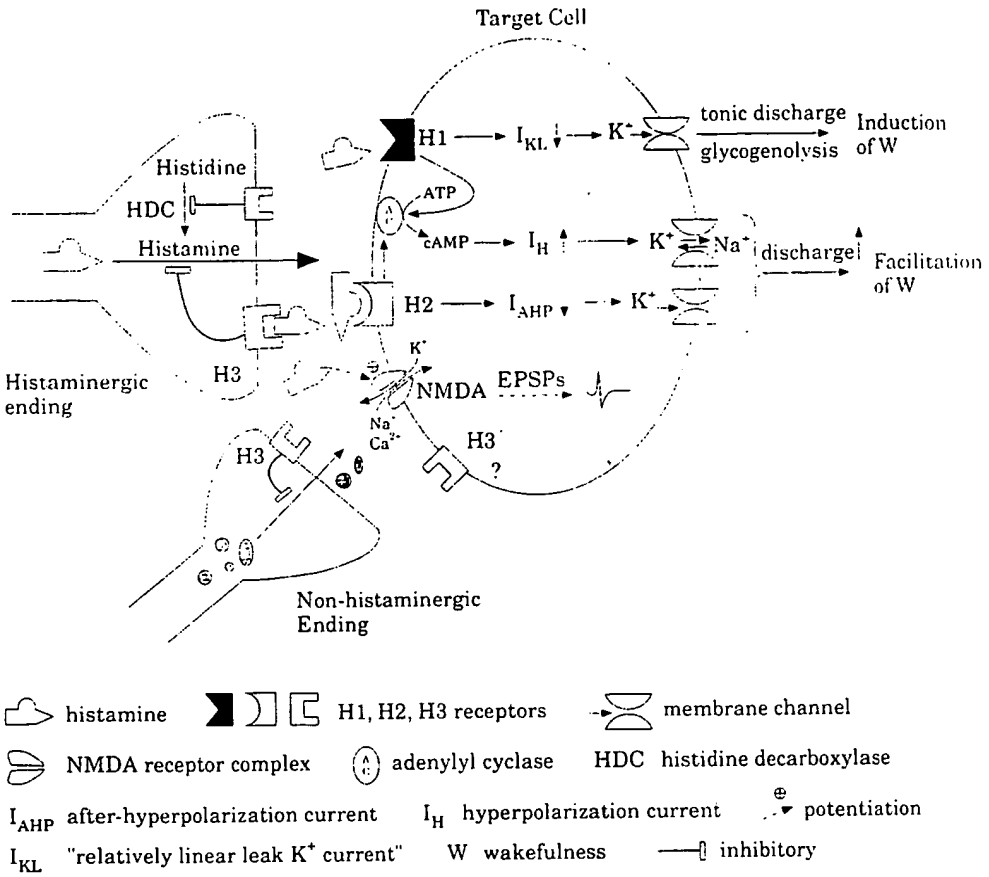

Figure 8 Schematic circuitry summarizing brain histaminergic transmission and current hypothesis concerning cellular mechanisms involved in histaminergic arousal (modified from Ref. 23). See text for comments.

transmission, e.g. by blocking its autoinhibition, promotes waking. These results, taken together with the recent *in vitro* electrophysiological data mentioned earlier, have led to a general hypothesis concerning cellular mechanisms involved in histaminergic arousal [23]. As schematically illustrated in Figure 8, this hypothesis can be summarized as follows. Activation of H1-receptors, by suppressing the leakage potassium current and by slow depolarization, would trigger the tonic neuronal discharge of large brain target cells and other physiological responses, such as glycogenolysis, required for high energy output, leading consequently to general brain arousal and increased vigilance. Activation of H2-receptors, on the other hand, by increasing neuronal excitability and discharge rate via hyperpolarization and afterhyperpolarization currents might facilitate cortical activation and waking. H1- and H2-receptors might therefore act in a synergic manner, through, respectively, direct neuronal depolarization or facilitation. Finally, H3-receptors are involved in the sleep–wake cycle by a negative control over histamine release and synthesis. The significance of the histamine-mediated enhancement of NMDA transmission [91, 92] in sleep–wake regulation remains to be investigated (Fig. 8).

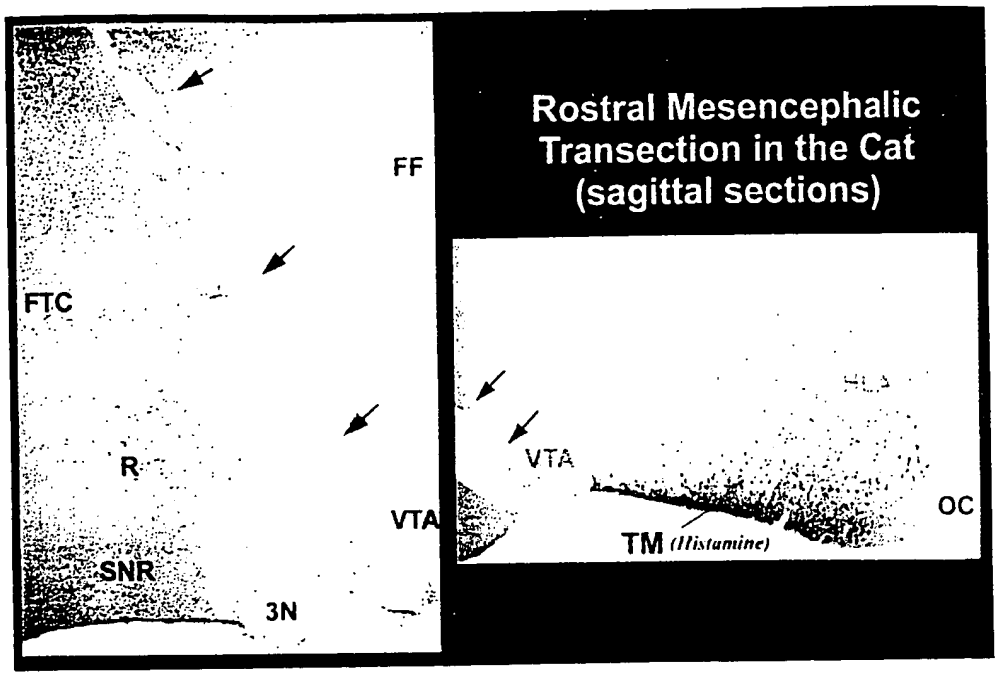

Figure 10 Photomicrographs of sagittal sections through the mesencephalon (left side) and hypothalamus (right side) showing the location of brain transection in the cat. At approximate the L 2.3 level, the transection (indicated by three arrows) is situated at the junction between the mesencephalon and hypothalamus, just rostral to the red nucleus (R) and the root of the third nerve (3 N). At a more lateral level (approximate L 2.5), the transection (indicated by two arrows) is just caudal to the histaminergic tuberomammillary (TM) nucleus, which remains intact. See comments in text. Other abbreviations: HLA, lateral hypothalamic area; FF, nucleus of the fields of Forel; FTC, central tegmental field; OT, optic tract; SNR, substantia nigra, reticular division; VTA, ventral tegmental area of Tsai (based upon work published by Lin *et al.* in Ref. 100 and author's unpublished data).

Hypothalamic "waking territory" and distribution of histaminergic cell bodies

As indicated above, inactivation of the posterior hypothalamus by muscimol induces hypersomnia in normal cats and restores sleep when animals are rendered insomniac. One question that could be raised is whether our muscimol injections have inactivated histaminergic neurons, thus causing an increase in SWS.

In the rat, the perikarya and dendrites of TM neurons are contacted synaptically by GABA terminals [48], and gabaergic afferents to histaminergic neurons have also been identified [54]. *In vitro* electrophysiological data also indicate tht histaminergic neurons possess gabaergic inputs and functional $GABA_A$-receptors [84]. Thus, application of GABA causes hyperpolarization of histaminergic neurons in a dose-dependent manner, the effect being prevented by bicuculline, an antagonist of $GABA_A$-receptors [84]. We have shown in the cat [64], that the hypothalamic "waking territory" (Fig. 9B), defined using muscimol (a potent $GABA_A$-receptor agonist), covers the middle and rostral parts of the posterior hypothalamus, including the TM nucleus and adjacent ventrolateral areas (Fig. 9A). Thus, it seems likely that the observed increase in SWS might

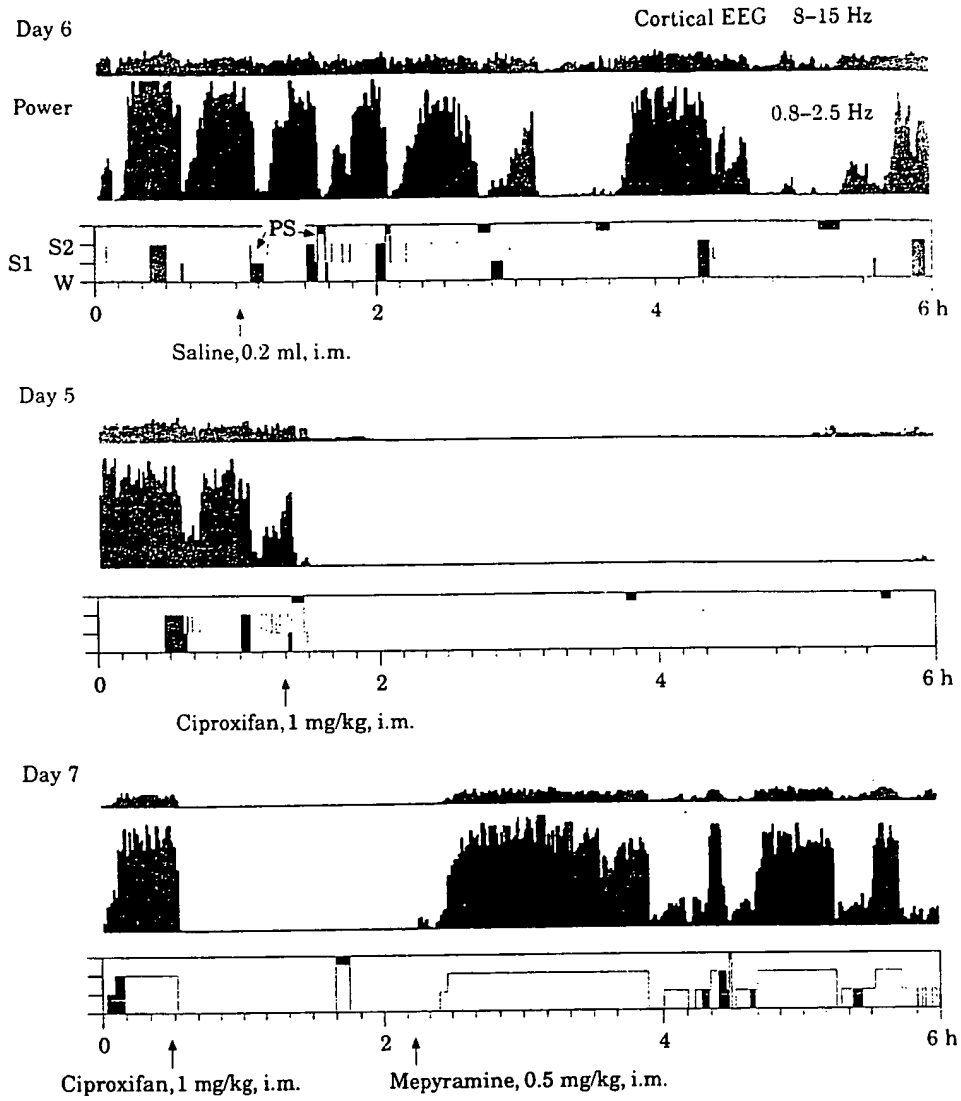

Figure 12 Effects of ciproxifan on the cortical EEG and "sleep–wake cycle" in the rostral mesencephalic transectioned cat and their reversibility by mepyramine. Representative examples of 6-h hypnograms and neocortical power spectra at 0.8–2.5 and 8–15 Hz showing the "sleep–wake cycle" and cortical activity (from top to bottom) on days 6 (upper panels, saline injection), 5 (middle panels, ciproxifan injection, 1 mg/kg), and 7 [lower panels, ciproxifan and mepyramine (0.5 mg/kg) injection] post-transection. See text for comments and Fig. 11 for abbreviations. X-axis: time in hours (based on work published by Lin *et al.* in Ref. 100 and author's unpublished data).

intact, while their descending axons and ascending projections from the brainstem are cut; Fig. 10), and examined the effects of ciproxifan on cortical EEG and expression of the immediate early gene, *c-fos*, an indicator of cellular activation. As mentioned above, ciproxifan is a new and potent H3-receptor antagonist that enhances the release and turnover of histaminergic neurons by a specific antagonism via autoinhibitory

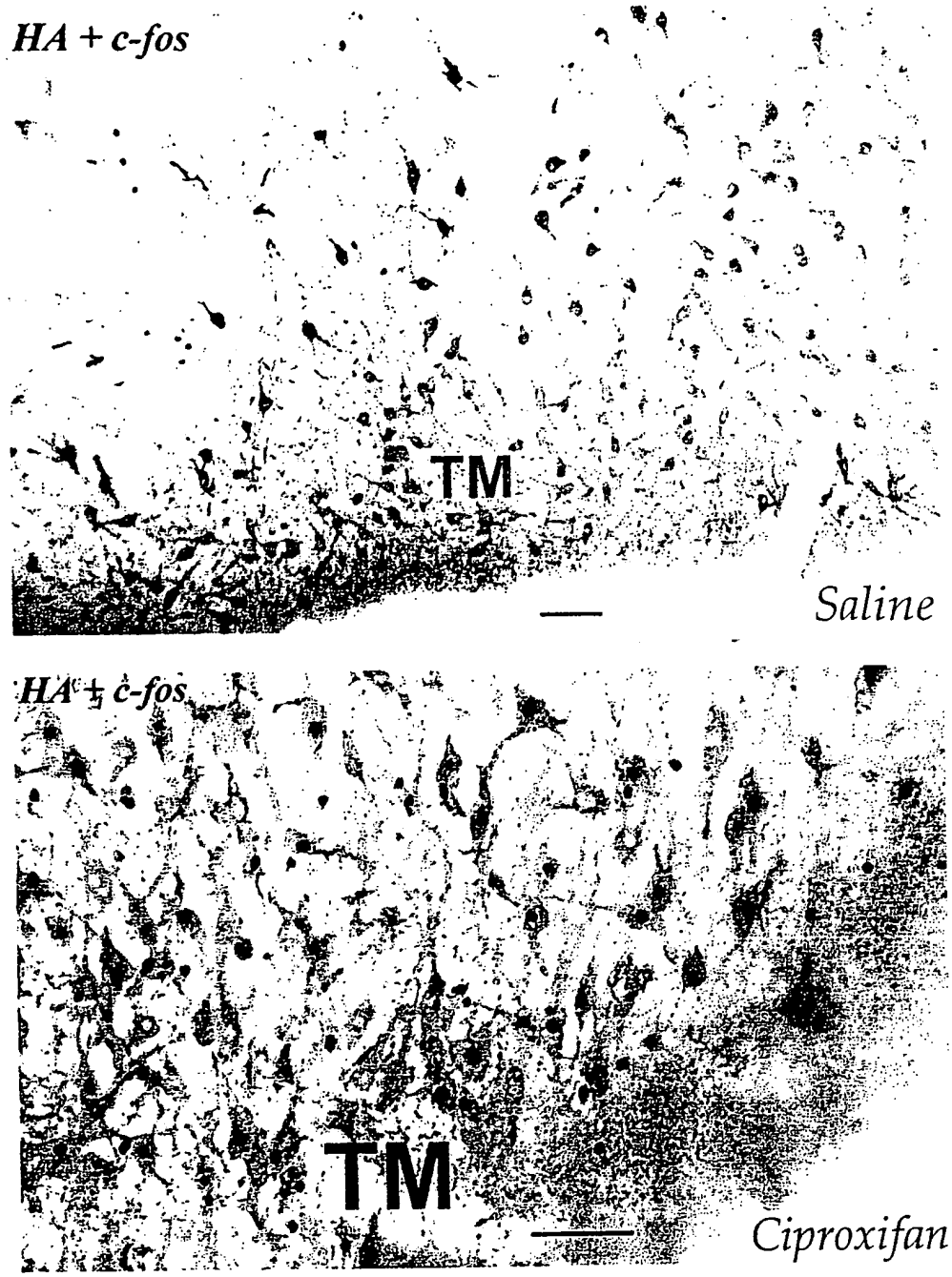

Figure 14 Photomicrographs of sagittal sections through the tuberomammillary nucleus (TM) showing *c-fos* and histamine (HA) immunoreactivity in the mesencephalic transectioned cat killed 1 h after intramuscular injection of saline or ciproxifan (1 mg/kg). Note that, after saline injection (upper section), only a few *c-fos* labelled cells (nuclear labelling in black) are seen in the HA-immunoreactive TM nucleus and adjacent region, while, after ciproxifan injection (lower section focussed on the rostral part of the TM), the great majority of HA-immunoreactive neurons (brown staining) also shows *c-fos* labelling. See details in text (bars = 50 μl, based on work published by Lin *et al.* in Ref. 100 and author's unpublished data).

antagonists) is able to enhance the release and turnover of histaminergic neurons via autoinhibitory mechanisms [99] and to cause their activation seen in the present study using *c-fos* might constitute a helpful experimental mean for their pharmacological identification when their unitary activity is recorded *in vivo*, a method that is lacking at present.

Hypothalamo-pre-optic histaminergic projections and their interaction with neurons in the pre-optic area

Among the different ascending pathways of histaminergic neurons involved in arousal, mention should be made of those projecting to the pre-optic/anterior hypothalamus. This region is considered to play an important role in sleep generation, mainly because its lesion, probably by destruction of neurons discharging in high rate during SWS, causes insomnia [22, 66]. The pre-optic area is probably the brain structure with the greatest density of histaminergic fibers, terminals and receptors. We have previously shown that the action of histamine in this region is to enhance wakefulness, probably by H2-receptors, and suggested that histamine cells can exert control over the pre-optic sleep-generating mechanisms via their projections to this structure [101]. Taken together with the fact that the insomnia induced by pre-optic lesion is reversed by inactivation of the ventrolateral posterior hypothalamus [66], these data indicate the importance of an intrahypothalamic interaction in the alternation of the sleep–wake cycle, an idea that is consistent with the long-standing hypothesis of Nauta [56], and suggest that this interaction in the caudo-rostral sense might be, at least in part, histaminergic. As regards interaction in the rostro-caudal sense, recent studies have indicated that GABA neurons in the pre-optic area send heavy projections to the posterior hypothalamus and TM histaminergic neurons (e.g. Ref. 54), and functional GABA innervation of histaminergic cells has been demonstrated [84]. Furthermore, electrical stimulation of the ventrolateral pre-optic area, in which neurons exhibit increased discharge during SWS and PS [102], induces hyperpolarization of TM neurons *in vitro* in brain slices [103]. Finally, as indicated in the above section, muscimol microinjection into the posterior hypothalamus, containing histamine cells, induces hypersomnia [64]. Taken together, all these results tend to suggest that the interactions between histamine and GABA neurons might constitute one of the important hypothalamic mechanisms underlying the alternation of sleep and wakefulness. Other sources of GABA in the posterior hypothalamus, such as GABA in the afferents from the mesopontine tegmentum and adjacent regions [53] and GABA from local interneurons, are also present, but their role in the inactivation of histamine cells during sleep and in sleep induction remains to be determined. Furthermore, the determination of the functional significance of the presence of GABA within histaminergic neurons, currently reported only in the rodent [48, 49], constitutes a challenge for our understanding of histaminergic transmission and neuronal functions.

Histaminergic ascending and descending projections and their interaction with cholinergic neurons

Among the histaminergic ascending pathways, those projecting to the basal forebrain and to the cholinergic neurons present in this region should also be noted. Our double

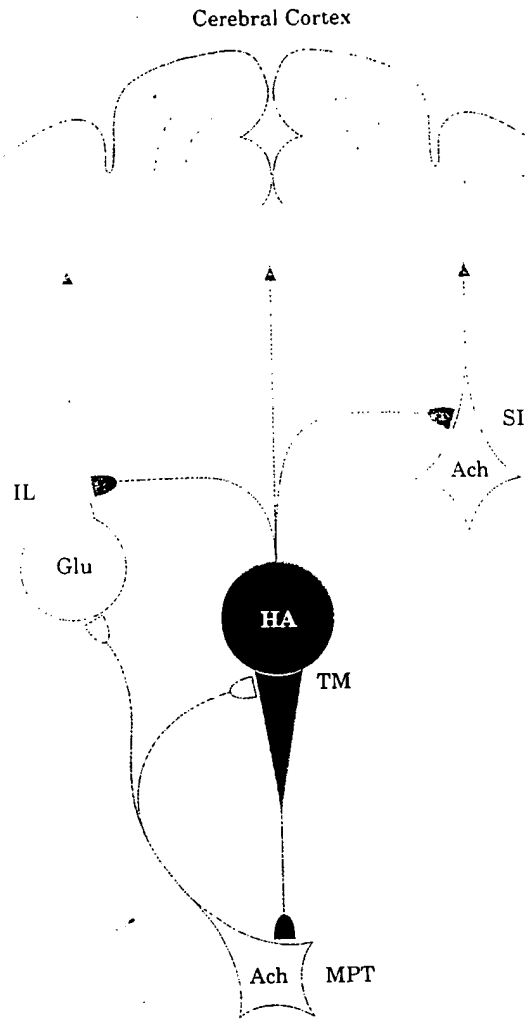

Figure 15 Schematic circuitry representing our hypothesis about the multiple interactions between histaminergic and cholinergic neurons involved in the neocortical activation of wakefulness. This hypothesis can be summarized as follows: neocortical activation during wakefulness is ensured by the excitatory corticopetal projections (arrows) arising mainly from three subcortical structures, the thalamus, posterior hypothalamus, and basal forebrain, and from brainstem aminergic neurons (not shown). These three subcortical systems are respectively represented by the presumed glutamatergic (Glu) intralaminar nuclei (IL), the histaminergic (HA) tuberomammillary nucleus (TM), and the cholinergic (Ach) substantia innominata (SI). The thalamic IL and hypothalamic TM nuclei receive ascending excitatory afferents from the mesepontine tegmentum (MPT) cholinergic neurons (Ach). Histaminergic neurons can activate the cortex either directly by their widespread hypothalamocortical projection, or indirectly via the thalamocortical system. In addition, they could promote cortical activation via the cholinergic system by a dual activation of the corticopetal system, originating from the SI, and of the pontothalamic or pontohypothalamic systems, arising from the MPT group. The excitatory interactions between histaminergic and cholinergic neurons in the MPT, posterior hypothalamus, and basal forebrain constitute a crucial circuit within the whole ascending network responsible for the maintenance of cortical activation and wakefulness [from Lin *et al.* (1996) with permission].

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CHAPTER 35

Histamine

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In a certain way, histaminergic systems have had a great, although indirect, historical importance in the development of neuropsychopharmacology. Indeed the discovery of both the neuroleptic and tricyclic antidepressant drugs in the 1950s was derived from the clinical study of behavioral actions of "antihistamines," a class of antiallergic drugs now designated H_1-receptor antagonists.

Nevertheless, the histaminergic neuronal system in brain, although already unraveled by the mid-1970s, has remained largely unexploited in drug design. Thus, only the traditional brain-penetrating H_1-receptor antagonists, used as over-the-counter sleeping pills, are known to interfere with histaminergic transmissions in the central nervous system (CNS). This contrasts with emergence, during the last decade, of a detailed knowledge of the system revealing that it shares many biological and functional properties with other aminergic systems overexploited in CNS drug design.

Histamine and its receptors in the brain have recently been the subject of two comprehensive reviews (22,62). Therefore, in order to limit the length of the present chapter we have deliberately selected to summarize the detailed information that can be found in these reviews, adding only more recent information and major references.

ORGANIZATION OF THE HISTAMINERGIC NEURONAL SYSTEM

One decade after the first evidence by Garbarg et al. of an ascending histaminergic pathway obtained by lesions of the medial forebrain bundle, the exact localization of corresponding perikarya in the posterior hypothalamus was revealed immunohistochemically. Since then, the distribution, morphology, and connections of histaminergic neurons have been determined. Data were recently reviewed (49,66,68,73) and will only be summarized briefly here.

All known histaminergic perikarya constitute a continuous group of mainly magnocellular neurons (about 2000 in the rat), located in the posterior hypothalamus and collectively named the *tuberomammillary nucleus.* It can be subdivided into medial, ventral, and diffuse subgroups extending longitudinally from the caudal end of the hypothalamus to the midportion of the third ventricle. A similar organization was described in humans, except that histaminergic neurons are more numerous (~64,000) and occupy a larger proportion of the hypothalamus (2). Besides their large size (25–35 μm), tuberomammillary neurons are characterized by few thick primary dendrites, with overlapping trees, displaying few axodendritic synaptic contacts. Another characteristic feature is the close contact of dendrites with glial elements in a way suggesting that they penetrate into the ependyma and come in close contact with the cerebrospinal fluid, perhaps to secrete or receive still unidentified messengers.

The histaminergic neurons are characterized by the presence of an unusually large variety of markers for other neurotransmitter systems: glutamic acid decarboxylase, the gamma-aminobutyric acid (GABA)-synthesizing enzyme; adenosine deaminase, a cytoplasmic enzyme possibly involved in adenosine inactivation; galanin, a peptide colocalized with all other monoamines; (Met^5) enkephalyl-Arg^6Phe^7, a product of the proenkephalin A gene; and other neuropeptides, such as substance P, thyroliberin, or brain natriuretic peptide. Tuberomammillary neurons also contain monoamine oxidase B, an enzyme responsible for deamination of tele-methylhistamine, a major histamine metabolite in brain. Finally, a subpopula-

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tion of histaminergic neurons is able to uptake and decarboxylate exogenous 5-hydroxytryptophan, a compound that they do not synthesize, however (66). Unraveling the functions of such a high number of putative co-transmitters in the same neurons remains an exciting challenge.

In analogy with other monoaminergic neurons, histaminergic neurons constitute long and highly divergent systems projecting in a diffuse manner to many cerebral areas. Immunoreactive, mostly unmyelinated, varicose or nonvaricose fibers are detected in almost all cerebral regions, particularly limbic structures. It was confirmed that individual neurons project to widely divergent areas (36). Ultrastructural studies suggest that these fibers make few typical synaptic contacts.

Fibers arising from the tuberomammillary nucleus constitute two ascending pathways: one laterally, via the medial forebrain bundle, and the other periventricularly. These two pathways combine in the diagonal band of Broca to project, mainly in an ipsilateral fashion, to many telencephalic areas—for example, in all areas and layers of the cerebral cortex, the most abundant projections being to the external layers. Other major areas of termination of these long ascending connections are the olfactory bulb, the hippocampus, the caudate putamen, the nucleus accumbens, the globus pallidus, and the amygdaloid complex. Many hypothalamic nuclei exhibit a very dense innervation—for example, the suprachiasmatic, supraoptic, arcuate, or ventromedial nuclei.

Finally, a long descending histaminergic subsystem arises also from the tuberomammillary nucleus to project to a variety of mesencephalic and brainstem structures such as the cranial nerve nuclei (e.g., the trigeminal nerve nucleus), the central gray, the colliculi, the substantia nigra, the locus coeruleus, the dorsal raphe nucleus, the cerebellum (sparse innervation), and the spinal cord.

Several anterograde tracing studies by Wouterloud and colleagues (73) established the existence of afferent connections to the histaminergic perikarya—namely, from the infralimbic division of the prefrontal cortex, the septum–diagonal band complex, the medial preoptic area, and the hippocampal area (subiculum).

MOLECULAR PHARMACOLOGY AND LOCALIZATION OF HISTAMINE RECEPTOR SUBTYPES

Three histamine receptor subtypes (H_1, H_2, and H_3) have been defined by means of functional assays and, subsequently, design of selective agonists and antagonists (22,63). All three seem to belong to the superfamily of receptors with seven transmembrane domains and coupled to guanylnucleotide-sensitive G proteins (Table 1) (see also Chapter 27, *this volume*).

The Histamine H_1 Receptor

The H_1 receptor was initially defined in functional assays (e.g., smooth muscle contraction) and the design of potent antagonists, the so-called "antihistamines" (e.g. mepyramine), most of which display prominent sedative properties.

Biochemical and localization studies of the H_1 receptor were made feasible with the design of reversible and irreversible radiolabeled probes such as [^{3}H]mepyramine, [^{125}I]iodobolpyramine, and [^{125}I]iodoazidophenpyramine (20,50,56).

Initial biochemical studies indicated that the cerebral guinea-pig H_1 receptor was a glycoprotein of apparent molecular size of 56 kD with critical disulfide bonds and that agonist binding was regulated by guanyl nucleotides, implying that the receptor belongs to the superfamily of receptors coupled to G proteins. In addition, various intracellular responses were found to be associated with H_1

TABLE 1. *Properties of three histamine receptor subtypes*

Property	H_1	H_2	H_3
Coding sequence	491 a.a. (bovine) 488 a.a. (guinea pig) 486 a.a. (rat)	358 a.a. (rat) 359 a.a. (dog, human)	?
Chromosome localization	Chromosome 3	?	?
Highest brain densities	Thalamus Cerebellum Hippocampus	Striatum Cerebral cortex Amydgala	Striatum Frontal cortex Substantia nigra
Autoreceptor	No	No	Yes
Affinity for histamine	Micromolar	Micromolar	Nanomolar
Characteristic agonists	2(*m*-chlorophenyl)histamine	Impromidine Sopromidine	(R)α-methylhistamine Imetit
Characteristic antagonists	Mepyramine (pyrilamine)	Cimetidine	Thioperamide
Radioligands	[^{3}H]Mepyramine [^{125}I]Iodobolpyramine	[^{3}H]Tiotidine [^{125}I]Iodoaminopotentidine	[^{3}H](R)α-methylhistamine [^{125}I]Iodophenpropit
Second messengers	Inositol phosphates (+) Arachidonic acid (+) cAMP (potentiation)	cAMP (+) Arachidonic acid (−) Ca^{2+} (+)	Inositol phosphates (−

receptor stimulation: inositol phosphate release, increase in Ca^{2+} fluxes, cyclic AMP or cyclic GMP accumulation in whole cells, and arachidonic acid release (22). It was not known, however, whether such a variety of responses corresponds to a single receptor or to distinct isoreceptors. Indeed, several photoaffinity-labeled proteins of slightly different sizes, but similar H_1 pharmacology, were detected in some tissues (56).

In spite of preliminary attempts using affinity columns with a mepyramine derivative, the H_1 receptor was never purified to homogeneity. Nevertheless, the deduced amino acid sequence of a bovine H_1 receptor was recently disclosed after expression cloning of a corresponding cDNA. The latter was based upon the detection of a Ca^{2+}-dependent Cl^- influx into microinjected *Xenopus* oocytes. Following the transient expression of the cloned cDNA into COS-7 cells, the identity of the protein as an H_1 receptor was confirmed by binding studies (75).

Starting from the bovine sequence, the H_1-receptor DNA was also cloned in the guinea pig (23,69), a species in which the pharmacology of the receptor is better established. Although marked species differences in H_1-receptor pharmacology had been reported (62), the sequence homology between the putative transmembrane domains (TMs) of the two proteins is rather high (90%).

In both species, characteristic amino acid residues thought to bind the histamine molecule at the level of the ammonium and imidazole groups are found: an aspartate (Asp^{116} in the guinea pig) in TM3, and a threonine (Thr^{203}) and an aspargine (Asn^{207}) in TM5, respectively. Also the "anatomy" of the H_1 receptor, with a long i_3 (third intracellular domain) and a short C-terminal tail, is similar to that of other receptors positively coupled to phospholipases A_2 and C. Amino acid sequence homology between the TMs of the H_1 and of the muscarinic receptors (~45%) is higher than between those of H_1 and H_2 receptors (~40%). H_1-receptor antagonists often display significant antimuscarinic activity but only limited H_2-receptor antagonist properties.

A single intronless gene seems to encode the guinea-pig H_1 receptor, and mRNAs of similar size were detected in brain areas and peripheral tissues (69). Thus the two pharmacologically indiscernible isoforms of the H_1 receptor of 56 and 68 kD, detected after photolabeling and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in some tissues (56), may correspond either to the same protein with different degrees of glycosylation or to the product of another similar gene revealed by Southern blot analysis (23).

When stably expressed in transfected fibroblasts, the guinea-pig H_1 receptor was found to trigger a large variety of intracellular signals involving or not coupling to pertussis-toxin-sensitive G proteins (Gi or Go)—namely, Ca^{2+} transients, inositol phosphates, or arachidonate release (33). H_1-receptor stimulation potentiates cAMP accumulation induced by forskolin in the same transfected fibroblasts, a response which resembles the H_1 potentiation of histamine H_2- or adenosine A_2-receptor-induced accumulation of cAMP in brain slices. All these responses mediated by a single H_1 receptor were known to occur in distinct cell lines or brain slices but could have been due to stimulation of isoreceptors.

The H_1 receptor mediates various excitatory responses in brain (21). In addition, in lateral geniculate relay neurons, it was recently shown to be responsible for a slow depolarization due to decrease in a K^+ current (39).

H_1-receptor distribution in the guinea-pig brain was established autoradiographically using [^{3}H]mepyramine or the more sensitive probe [^{125}I]iodobolpyramine (50) and the information complemented by in situ hybridization of the mRNA (23,69). For instance, the high density of H_1 receptors in the molecular layers of cerebellum and hippocampus seems to correspond to dendrites of Purkinje and pyramidal cells, respectively, in which the mRNA is highly expressed. H_1 receptors are also abundant in guinea-pig thalamus, hypothalamic nuclei (e.g., ventromedial nuclei), nucleus accumbens, amygdaloid nuclei, and frontal cortex but not in neostriatum (50), whereas they are more abundant in the human neostriatum (37).

The H_1 receptor was visualized in the primate and human brain by positron emission tomography using [^{11}C]-mepyramine (76).

The widespread distribution of the H_1 receptor in cerebral areas involved in wakefulness and cognition presumably accounts for the sedative properties of "antihistamines" of the first generation.

The Histamine H_2 Receptor

Molecular properties of the H_2 receptor have remained largely unknown for a long time. For instance, reversible labeling of the H_2 receptor was achieved only recently using [^{3}H]tiotidine or, more reliably, [^{125}I]-iodoaminopotentidine (62). Irreversible labeling, using a photoaffinity probe, followed by SDS-PAGE led to the identification of H_2-receptor peptides from the guinea pig (56).

By screening cDNA or genomic libraries with homologous probes, the gene encoding the H_2 receptor was first identified in dogs (18) and, subsequently, in rats (55) and humans (17). The H_2 receptor is organized like other receptors positively coupled to adenylyl cyclase; that is, it displays a short third intracellular loop and a long C-terminal cytoplasmic tail.

Initially, binding of the histamine molecule in the H_2 receptor seemed to involve (a) the carboxylate of Asp^{98} in TM3 as a counterion for the ammonium and (b) Asp^{186} and Thr^{190} for proton transfer and hydrogen bonding with the imidazole ring, but this idea found only partial support from site-directed mutagenesis (16).

Using transfected cell lines, not only the well-estab-

lished positive linkage of the H_2 receptor with adenylyl cyclase (28) was confirmed, but also the unexpected inhibition of arachidonate release (71) and stimulation of Ca^{2+} transients (13). Hence H_2-receptor stimulation can trigger intracellular signals either opposite or similar to those evoked by H_1-receptor stimulation. Parallel observations were made for a variety of biological responses mediated by the two receptors in peripheral tissues.

Helmut Haas and colleagues showed that, in hippocampal pyramidal neurons, H_2-receptor stimulation potentiates excitatory signals by decreasing a Ca^{2+}-activated K^+ conductance, presumably via cAMP production (21). H_2-receptor activation depolarizes thalamic relay neurons slightly, increasing markedly apparent membrane conductance, a response due to enhancement of the hyperpolarization-activated cation current I_h (39).

The sole selective H_2-receptor antagonist known to enter the brain is zolantidine, a compound used sometimes in animal behavioral studies but never introduced in therapeutics (15). However, a number of tricyclic antidepressants are known to block H_2-receptor-linked adenylylcyclase quite potently and interact with [^{125}I]-iodoaminopotentidine binding in a complex manner (72).

Autoradiographic localization of H_2 receptors in guinea pig performed using [^{125}I]iodoaminopotentidine shows them distributed heterogeneously in a manner suggesting their major association with neurons (50). H_2 receptors are found in most areas of the cerebral cortex, with the highest density in the superficial layers, the piriform and occipital cortices, which contain low H_1-receptor density. The caudate putamen, ventral striatal complex, and amygdaloid nuclei (bed nucleus of the stria terminalis) are among the richest brain areas. In the hippocampal formation, H_2 receptors display a laminated pattern with labeling of lacunosum moleculare, radiatum, and oriens layers; the partial overlap with H_1 receptors may account for their synergistic interaction in cAMP accumulation. H_2 receptors in human brain were characterized and localized using [^{125}I]iodoaminopotendine (70).

The Histamine H_3 Receptor

The H_3 receptor was initially detected as an autoreceptor controlling histamine synthesis and release in brain. Thereafter it was shown to inhibit presynaptically the release of other monoamines in brain and peripheral tissues as well as of neuropeptides from unmyelinated C fibers (3).

The molecular structure of the H_3 receptor remains to be established. Reversible labeling of this receptor was first achieved using the highly selective agonist [^{3}H](R)α-methylhistamine (62); then [^{3}H]*N*α-methylhistamine, a less selective agonist, was also proposed (20) as well as, more recently, [^{125}I]iodophenpropit, an antagonist (27). The binding of [^{3}H](R)α-methylhistamine is regulated by guanyl nucleotides, strongly suggesting that the H_3 receptor, like the other histamine receptors, belongs to the superfamily of receptors coupled to G proteins (62). Constitutive H_3 receptors in a gastric cell line appear to be negatively coupled to phospholipase C via a mechanism sensitive to both pertussis and cholera toxins (6). In contrast, H_3 receptors in vascular smooth muscle mediate voltage-dependent Ca^{2+}-channel stimulation via a pertussis-insensitive G protein (44).

Recently, two highly potent and selective H_3-receptor agonists—(R)α,(S)β-dimethylhistamine (35) and imetit (19)—were designed. Like (R)α-methylhistamine, they are able to decrease brain histamine synthesis and release after systemic administration in low dosage (3,19,62). Thioperamide, a systemically active H_3 antagonist, markedly increases histamine turnover in brain (3) and, because no other class of drug is available for this purpose, is widely used in behavioral studies.

Functional studies have evidenced inhibitory H_3 receptors on nerve terminals not only of histaminergic (3,62) but also noradrenergic (58), serotoninergic (14), dopaminergic (59), cholinergic (7), and peptidergic neurons (38).

Autoradiography of H_3 receptors in rat (12,51) and monkey brain (37) shows them highly concentrated in neostriatum, nucleus accumbens, cingulate and infralimbic cortices, bed nucleus of stria terminalis, and substantia nigra pars lateralis. In contrast, their density is relatively low in the hypothalamus (including the tuberomammillary nucleus), which contains the highest density of histaminergic axons (and perikarya), indicating that the majority of H_3 receptors are not autoreceptors. In agreement, intrastriatal kainate strongly decreases H_3 binding sites in forebrain (as well as in substantia nigra, consistent with their presence in striatonigral neurons) (12,51).

HISTAMINERGIC NEURON ACTIVITY AND THEIR CONTROL

Electrophysiological Properties

Cortically projecting histaminergic neurons share with other aminergic neurons a number of electrophysiological properties evidenced by extracellular recording. They fire spontaneously slowly and regularly, and their action potentials are of long duration (21). In addition, they exhibit inward rectification attributed to an I_h current that may increase whole-cell conductance and decrease the efficacy of synaptic inputs during periods of prolonged hyperpolarization (see Chapter 5, *this volume*)—that is, when histaminergic neurons fall silent (29).

Modulation of Histamine Synthesis and Release In Vitro

The autoreceptor-regulated modulation of histamine synthesis in, and release from, brain neurons is now well-

cumented (62). It was initially evidenced in brain slices synaptosomes after labeling the endogenous pool of stamine using the ^3H precursor. Exogenous histamine creases the release and formation of [^{3}H]histamine inced by depolarization, and analysis of these responses l to the pharmacological definition of H_3 receptors. The toregulation was found in various brain regions known contain histamine nerve endings, suggesting that all minals are endowed with H_3 autoreceptors. A regulan of histamine synthesis was also observed in the poster hypothalamus, possibly indicating the existence of toreceptors at the level of histaminergic perikarya or ndrites (3).

Galanin, a putative co-transmitter of a subpopulation histaminergic neurons, regulates histamine release only regions known to contain efferents of this subpopulan—that is, in hypothalamus and hippocampus but not cerebral cortex or striatum (4). In brain slices, galanin so hyperpolarizes and decreases the firing rate of tuberoammillary neurons (60). It is not known, however, hether these galanin "autoreceptors" modulate galanin lease from histaminergic nerve terminals. Other putae co-transmitters of histaminergic neurons failed to fect [^{3}H]histamine release from slices of rat cerebral rtex (61).

[^{3}H]Histamine synthesis and release are inhibited in rious brain regions by stimulation of not only autoreptors but also α_2-adrenergic receptors, M_1-muscarinic ceptors, and κ-opioid receptors (62). Muscarinic receprs also inhibit endogenous histamine release in hypoalamus (48). Since these regulations are also observed ith synaptosomes (61), all these receptors presumably present true presynaptic heteroreceptors. In contrast, stamine release is enhanced by stimulation of nicotinic ceptors in rat hypothalamus (48) and by μ-opioid receprs in mouse cerebral cortex (62).

hanges in Histaminergic Neuron Activity In Vivo

Both neurochemical and electrophysiological studies dicate that the activity of histaminergic neurons is high ring arousal. In rat hypothalamus, histamine levels are w whereas synthesis is high during the dark period, ggesting that neuronal activity is enhanced during the tive phase (62). Histamine release from the anterior pothalamus of freely moving rats, evaluated by in vivo icrodialysis, gradually increases in the second half of e light period and is maintained at a maximal level ring the active phase (40). Such state-related changes e also found in single-unit extracellular recordings perrmed in the ventrolateral posterior hypothalamus of eely moving cats. Neurons with properties consistent ith those of histaminergic neurons exhibited a circadian ythm of their firing rate, falling silent during deep slowave or paradoxical sleep (62).

A feeding-induced increase in the activity of histaminergic neurons has also been shown by microdialysis performed in the hypothalamus of conscious rats (26). Changes in the metabolism and release of histamine observed in vivo after occlusion of the middle cerebral artery in rats suggest that the histaminergic activity is also enhanced by cerebral ischemia (1).

Whereas H_1 and H_2 receptors are apparently not involved, inhibition mediated by H_3 autoreceptors constitutes a major regulatory mechanism for histaminergic neuron activity under physiological conditions. Administration of selective H_3-receptor agonists reduces histamine turnover (62) and release, as shown by microdialysis (24). In contrast, H_3-receptor antagonists enhance histamine turnover (62) and release in vivo (25,41), suggesting that autoreceptors are under tonic stimulation by endogenous histamine.

Agents inhibiting histamine release in vitro via stimulation of presynaptic α_2-adrenergic or muscarinic heteroreceptors reduce histamine release and turnover in vivo, but systemic administration of antagonists of these receptors does not generally enhance histamine turnover, suggesting that heteroreceptors are not tonically activated under basal conditions (9,45,52,61).

Activation of central nicotinic (45), 5-HT_{1A} serotonergic (46), and dopaminergic receptors (53) inhibits histamine turnover, but the presynaptic location of these receptors remains to be demonstrated. Histamine turnover in the brain is also rapidly reduced after administration of various sedative drugs such as ethanol, Δ^9-tetrahydrocannabinol, barbiturates, and benzodiazepines (62). The effect of the latter compounds may result from their interaction in vivo with GABA receptors present on nerve endings of a subpopulation of histaminergic neurons containing GABA (66).

In contrast, stimulation of μ-opioid (62) and *N*-methyl-D-aspartate (NMDA) receptors (47) enhances histamine release and turnover in brain. Morphine increases histamine release in the periaqueductal gray (5).

The effect of reserpine on brain histamine turnover appears to be controversial: Both enhancement (62) and inhibition (42) were reported.

PHYSIOLOGICAL ROLES OF HISTAMINERGIC NEURONS

In spite of many different suggestions mainly derived from the observations of responses to locally applied histamine, only few physiological roles of histaminergic neurons appear relatively well-documented.

Arousal

Following our initial proposal in 1977, a large body of experimental evidence has accumulated to indicate that

histaminergic neurons play a critical role in cortical activation and arousal mechanisms (62).

Intracerebral injection of histamine, particularly in the cat ventrolateral hypothalamus, where the density of histaminergic axons is high, increases wakefulness via stimulation of postsynaptic H_1 receptors. Endogenous histamine presumably plays a similar role because inhibition of its synthesis by an L-histidine decarboxylase inhibitor, inhibition of its release by an H_3-receptor agonist, and inhibition of its action by an H_1-receptor antagonist all increase deep slow-wave sleep and decrease wakefulness in several animal species; conversely, inhibitors of histamine methylation or H_3-receptor antagonists, which facilitate the amine release, both increase arousal (34,62). The role of histaminergic neurons in arousal is also shown by the decreased wakefulness following lesions of the posterior hypothalamus, particularly those aimed at destruction of the tuberomammillary nucleus.

Finally, histaminergic neurons share with other cortically projecting aminergic neurons which control behavioral states a number of electrophysiological properties, including increased activity during wakefulness (see preceding section).

Cellular modes of action of histamine mediated by H_1 and H_2 receptors may well account for its "arousing effect." On thalamic relay neurons, histamine, acting through these receptors, exerts a double depolarizing action and thereby facilitates a change of neuronal activity from one of endogenous oscillation and poor responsiveness to sensory inputs which predominates during sleep, to one dominated by single-spike activity and a more accurate and faithful relay of sensory information which characterizes arousal (67). In addition, histamine will facilitate further processing of sensory information in the neocortex through the reduction of spike frequency adaptation resulting from a block of a Ca^{2+}-activated K^+ current mediated by the H_2 receptor (21). These various actions seem shared by other neurotransmitters (including acetylcholine, noradrenaline, serotonin, and glutamate) which collectively innervate the forebrain and, possibly via shifts in oscillatory states of thalamocortical networks, promote the characteristic changes in firing occurring between sleep and arousal (67).

All these cellular modes of action of histamine, taken together with observations indicating that its release from activated tuberomammillary neurons is maximal during wakefulness, suggest that the histaminergic systems make an important contribution to the ascending control of arousal, attention, sensory information processing, and cognition.

Accordingly, in humans, many H_1-receptor antagonists induce drowsiness, impair performances requiring attention, increase the tendency to sleep, and are ingredients of over-the-counter sleeping pills. These sedative effects are stereoselective (43). Conversely, a new generation of "antihistamines," unable to block cerebral H_1 receptors, are devoid of sedative properties. A rather large number of antidepressant (e.g., mianserin or doxepin) and antipsychotic agents (e.g., clozapine) display high H_1-receptor antagonist potency which presumably accounts for their sedative side effects (54).

Control of Pituitary Hormone Secretion

Exogenous and, in some cases, endogenous histamine were shown to affect the secretion of both posterior and anterior pituitary hormones (32,62).

Supraoptic nucleus neurons are typically excited by application of histamine, their firing rate being increased during secretory bursts of activity and the depolarizing afterpotential being enhanced (65). This effect is mediated by H_1 receptors and causes circulating vasopressin levels to rise. Histaminergic neurons may participate in the physiological control of vasopressin secretion because inhibition of histamine synthesis impairs the vasopressin response to adrenalectomy and stimulation of the tuberomammillary nucleus causes phasic supraoptic nucleus neurons to become more excitable (77). This control, however, may not be a tonic one because H_1-receptor antagonists do not appear to modify vasopressin secretion. It is not known whether the increase in water consumption elicited by an H_3-receptor agonist is related to an effect on supraoptic nucleus neurons (8).

Endogenous histamine may be involved in stress-, estrogen- or morphine-induced release of prolactin because the response is prevented by blockade of histamine synthesis or of postsynaptic H_1 or H_2 receptors or activation of presynaptic H_3 autoreceptors (32). Being also prevented by antibodies to vasopressin, the action of histamine may involve this neuropeptide. Endogenous histamine, acting primarily at H_1 receptors, may also be involved in the secretory responses of adrenocorticotropic hormone and β-endorphin induced by restraint or insulin hypoglycemia.

Although exogenous histamine predominantly inhibits the release of growth hormone and thyroid-stimulating hormone, the role of histaminergic neurons in the control of these hormones secretion is not established.

Control of Appetite

Weight gain is often experienced by patients receiving H_1 antihistamines or tricyclic antidepressants displaying potent H_1-receptor antagonist properties. It is likely that this reflects an inhibitory role of histamine neurons projecting to the ventromedial and paraventricular hypothalamic nuclei on feeding as shown by the effects of histamine synthesis inhibitors or H_3-receptor ligands (57). In addition, the extracellular concentration of the amine in rat hypothalamus increases during feeding (26).

;ulation of Seizure Susceptibility

he action of drugs affecting histamine synthesis or hylation, as well as of H_1- and H_3-receptor antagonists, gests that endogenous histamine may restrict the mani- ations of electrically and pentetrazole-induced sei- :s in rodents (78,79).

;ulation of Vestibular Reactivity

ome H_1 antagonists are the most commonly used anti- tion-sickness drugs, but it is not clear whether this is ıted to blockade of H_1 receptors. Histamine depolarizes rons in vestibular nuclei and modulates quite effec- :ly static vestibular reflexes through interaction with h H_2 and H_3 receptors (64,74). A beneficial effect of receptor antagonists in vertigo or motion sickness was ;gested by these data.

) HISTAMINERGIC NEURONS HAVE A)LE IN NEUROPSYCHIATRIC DISEASES?

Among the various approaches that tend to establish : implication of other aminergic neuronal systems in ıropsychiatric diseases, so far only a few were (or could) applied to histamine.

Postmortem studies in basal ganglia of patients with rkinson's disease (62) or in a rodent model of this ;ease (10) show no change in the activity of the hista- ne-synthesizing enzyme.

In the hypothalamus of Alzheimer's disease patients, ımerous neurofibrillary tangles and typical senile aques were detected in the tuberomammillary area; it as not clear, however, whether the number of histamine- ımunoreactive neurons was decreased (2). Because re-)rts on histamine levels in such patients have been con- cting, a role of histamine in the etiopathology of lzheimer's disease remains doubtful. In addition, it may : of significance that 9-amino-1,2,3,4-tetrahydroacridine THA), an anticholinesterase drug which was found use- ıl in Alzheimer's disease, is also a rather potent inhibitor f histamine methylation (11).

The effects of antipsychotics at dopamine receptors rongly suggested the role of dopamine in schizophrenia. ı contrast, the interaction of psychotropic drugs with istamine receptors are of limited help to deduce a role f histaminergic neurons in psychiatry. Over a decade go, the cerebral H_2 receptor was proposed by J. P. Green, . Greengard, and colleagues to represent an important ırget for most tricyclic and other antidepressant drugs ıat interact with relatively high affinity with the receptor oupled to the cyclase (54). The strong dependence of ıe apparent affinity of these compounds for the H_2 recep-)r upon the experimental conditions of the assay leaves ome doubt, however, about the therapeutic significance of this observation (72). It remains that a number of side effects of several antidepressant drugs as well as some neuroleptics (e.g., sedation or weight gain) are attributable to the blockade of cerebral H_1 receptors (54).

Unfortunately, the effects of drugs able to stimulate the three cerebral histamine receptor subtypes or to block H_2 or H_3 receptors in neuropsychiatric diseases are not known. Therefore, these receptors remain important potential targets for novel classes of psychotropic agents, particularly "cognition/arousal enhancers" acting via facilitation of histaminergic neurotransmission in brain.

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CHAPTER 35

Histamine

Jean-Charles Schwartz, Jean-Michel Arrang, Monique Garbarg, and Elisabeth Traiffort

In a certain way, histaminergic systems have had a great, although indirect, historical importance in the development of neuropsychopharmacology. Indeed the discovery of both the neuroleptic and tricyclic antidepressant drugs in the 1950s was derived from the clinical study of behavioral actions of "antihistamines," a class of antiallergic drugs now designated H_1-receptor antagonists.

Nevertheless, the histaminergic neuronal system in brain, although already unraveled by the mid-1970s, has remained largely unexploited in drug design. Thus, only the traditional brain-penetrating H_1-receptor antagonists, used as over-the-counter sleeping pills, are known to interfere with histaminergic transmissions in the central nervous system (CNS). This contrasts with emergence, during the last decade, of a detailed knowledge of the system revealing that it shares many biological and functional properties with other aminergic systems overexploited in CNS drug design.

Histamine and its receptors in the brain have recently been the subject of two comprehensive reviews (22,62). Therefore, in order to limit the length of the present chapter we have deliberately selected to summarize the detailed information that can be found in these reviews, adding only more recent information and major references.

ORGANIZATION OF THE HISTAMINERGIC NEURONAL SYSTEM

One decade after the first evidence by Garbarg et al. of an ascending histaminergic pathway obtained by lesions of the medial forebrain bundle, the exact localization of corresponding perikarya in the posterior hypothalamus was revealed immunohistochemically. Since then, the distribution, morphology, and connections of histaminergic neurons have been determined. Data were recently reviewed (49,66,68,73) and will only be summarized briefly here.

All known histaminergic perikarya constitute a continuous group of mainly magnocellular neurons (about 2000 in the rat), located in the posterior hypothalamus and collectively named the *tuberomammillary nucleus*. It can be subdivided into medial, ventral, and diffuse subgroups extending longitudinally from the caudal end of the hypothalamus to the midportion of the third ventricle. A similar organization was described in humans, except that histaminergic neurons are more numerous (~64,000) and occupy a larger proportion of the hypothalamus (2). Besides their large size (25–35 μm), tuberomammillary neurons are characterized by few thick primary dendrites, with overlapping trees, displaying few axodendritic synaptic contacts. Another characteristic feature is the close contact of dendrites with glial elements in a way suggesting that they penetrate into the ependyma and come in close contact with the cerebrospinal fluid, perhaps to secrete or receive still unidentified messengers.

The histaminergic neurons are characterized by the presence of an unusually large variety of markers for other neurotransmitter systems: glutamic acid decarboxylase, the gamma-aminobutyric acid (GABA)-synthesizing enzyme; adenosine deaminase, a cytoplasmic enzyme possibly involved in adenosine inactivation; galanin, a peptide colocalized with all other monoamines; (Met^5) enkephalyl-Arg^6Phe^7, a product of the proenkephalin A gene; and other neuropeptides, such as substance P, thyroliberin, or brain natriuretic peptide. Tuberomammillary neurons also contain monoamine oxidase B, an enzyme responsible for deamination of tele-methylhistamine, a major histamine metabolite in brain. Finally, a subpopula-

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Best Season's Greetings
Giulio

REVIEW ARTICLE

An Update on Histamine H_3 Receptors and Gastrointestinal Functions

GIULIO BERTACCINI, MD, PhD and GABRIELLA CORUZZI, PhD

The distribution and functions of histamine H_3 receptors in the gastrointestinal tract is reviewed with particular reference to the effects on gastric acid secretion, mucosal protection, and intestinal motility. Histamine H_3 receptor activation has negative effects on acid secretion induced by indirect secretagogues in cats, dogs, and rabbits; less clear effects were found in rats. An inhibitory effect on histamine release induced by different stimuli was observed in rats, rabbits, and dogs after H_3 receptor agonists, thus supporting the idea that H_3 receptors occur in ECL cells. (*R*)-α-methylhistamine has a marked protective effect against gastric lesions induced by ethanol in rats, being slightly less effective against aspirin and stress. H_3 receptor activation decreases the intestinal motility induced by electrical stimulation in a variety of gut preparations, reducing both cholinergic and NANC neurotransmitter release. In this tissue the inhibitory effects mediated by histamine H_3 receptors seem to be coupled, via a G protein, to a restriction of Ca^{2+} access into the nerve terminal; other mechanisms, however, have been suggested in the gastric mucosa. Histamine H_3 receptors have already been subdivided into two receptor subtypes, H_{3A} and H_{3B}, the former being the subtype predominant in the gastrointestinal tissue. The increasing availability of selective agonists and antagonists of H_3 receptors will unravel possible novel actions and physiological roles of histamine.

KEY WORDS: histamine H_3 receptors; gastric acid secretion; mucosal protection; intestinal motility.

With the discovery of histamine H_2 receptors by Sir James Black et al (1) and the consequent appearance in the literature of thousands of papers concerning H_2 antagonists, the study of histamine in the gastrointestinal tract seemed to be concluded. However, several areas of uncertainty still existed, and new horizons were opened up when a new subtype of histamine receptors, namely H_3, was described (2). H_3 receptors were initially found in the central nervous system, where, by acting as prejunctional autoreceptors, they negatively regulate histamine synthesis and release from histaminergic neurons. In subsequent studies, strong evidence has been presented that the histamine H_3 receptor is not restricted to histaminergic nerve terminals but also occurs as a heteroreceptor both in central and peripheral neurons, where it regulates the release of several neurotransmitters (noradrenaline, acetylcholine, 5-hydroxytryptamine, dopamine, etc) (for review see 3–6). H_3 receptors occur also in sensory neurons, where they inhibit the release of neuropeptides from C fibers, and in nonneural tissues, such as mast cells and ECL cells (7).

The experimental research on histamine was greatly influenced by the new discovery, and all histaminologists were pushed to reconsider their own work. In particular, investigators involved in gastrointestinal research had the opportunity to revisit all the problems which still remained unsolved, even after the H_2 antagonists era. In fact, although histamine was considered the main physiological mediator in gastric acid secretion, histamine release from gas-

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tric mucosal stores still remains the subject of strong debate, partly because cellular stores for histamine are different in the different species (8) and partly because the interplay of the endogenous mediators in gastric mucosa is very complex (9–11). Another central problem is the role of histamine in mucosal protection: many authors (12–14) have described histamine as an ulcerogenic substance, whereas, at the same time, others have claimed that histamine is actually a gastroprotective compound (15. 16).

As for intestinal motility, some doubts concern the so-called "anomalous" H_2 receptors stimulated by the H_2 agonists but not inhibited by the H_2 antagonists (17–19).

The present review will focus on H_3 receptors and gastrointestinal functions, with particular reference to acid secretion, mucosal protection, and intestinal motility.

CHEMISTRY OF H_3 RECEPTOR LIGANDS

As in the case of H_2 ligands, the synthesis of H_3 receptor agonists and antagonists started from histamine. Histamine itself activates H_3 receptors with a potency actually higher than that exerted at H_1 and H_2 receptors. The first H_3 antagonists were searched in the field of already known H_1 and H_2 ligands, and indeed burimamide and impromidine were found to possess rather high affinity for H_3 receptors (2), although they are nonselective compounds. In this respect, it must be emphasized that some other H_2 antagonists (ie, metiamide and mifentidine) have approximately the same affinity at H_2 and H_3 receptors (20); this casts some doubts on previous studies in which histamine-mediated responses were classified as H_2-mediated only on the basis of their blockade by these compounds. Starting from 1987 (21) highly potent and selective H_3 ligands were available, like (*R*)-α-methylhistamine as an agonist and thioperamide as an antagonist, and this has allowed the identification of H_3 receptors in a variety of tissues. In subsequent studies, a series of newly synthesized molecules were found to have a better potency and selectivity at histamine H_3 receptors (for review see 20, 22–26). In the field of agonists, most compounds are substitution products of histamine and most of the H_3 antagonists are imidazole derivatives. There are a few nonimidazole compounds that possess H_3 receptor activity, like dimaprit (20), betahistine (3), the psychotomimetic drug phencyclidine (27), and the antipsychotic drug clozapine (28); however, their potency at H_3 receptors is much lower than that of thioperamide. Tables 1 and 2 are a synopsis of the different H_3 ligands so far available for pharmacological studies. Their activity was determined in functional experiments from brain and intestinal tissues, considered as screening tests for the pharmacological evaluation of new H_3 ligands (29).

TISSUE DISTRIBUTION OF H_3 RECEPTORS

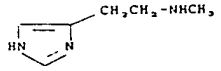
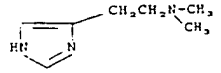
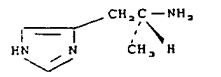
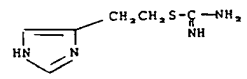
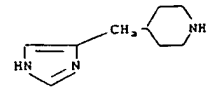
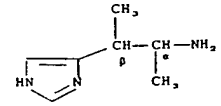
The availability of radiolabeled [^{3}H](*R*)-α-methylhistamine has allowed the study of the distribution of H_3 receptors in the different tissues. Binding and autoradiographic studies in rat and human brain showed that the distribution of H_3 receptors is highly heterogeneous (30, 31). The mean density of these receptors is rather low compared with that of H_1 and H_2 receptors and particularly in peripheral tissues (32). As regards the gastrointestinal tract, contrasting findings have been reported by the use of [^{3}H]N^{α}-methylhistamine (32) or [^{3}H]*S*-methylthioperamide (33) as a radioligand, the H_3 receptor density being lower or higher, respectively, in the stomach than in the large intestine. However, both these ligands were found to interact with non-H_3 receptor sites; thus radioligands with higher selectivity could represent more suitable probes. Recently, two radiolabeled antagonists, [^{125}I]iodophenpropit and [^{125}I]iodoproxyfan (34, 35) were synthesized and proved to be very potent and selective at histamine H_3 receptors; they could be therefore very useful for a definite assessment of the relative distribution of these receptors in the different tissues.

GASTRIC ACID SECRETION

In Vivo Experiments

The early *in vivo* experiments were performed in conscious cats fitted with gastric fistula and/or a Heidenhain pouch (36, 37). They were carried out by the use of (*R*)-α-methylhistamine, which proved to be a good inhibitor of gastric secretion induced by a number of indirect stimuli (2-deoxy-D-glucose (2-DG), pentagastrin, food, and bombesin). The inhibitory effect of (*R*)-α-methylhistamine, which never exceeded 50–60%, was counteracted by thioperamide, and this represented the first evidence of the involvement of H_3 receptors. Furthermore, thioperamide alone was able to enhance the stimulatory effect of submaximal doses of 2-DG and pentagastrin. This suggested that H_3 receptors might have a role in the

TABLE 1. AGONISTIC ACTIVITY OF SOME H_3 LIGANDS DETERMINED ON RAT CEREBRAL CORTEX AND GUINEA PIG INTESTINE

Compound	Structure	pD_2 values (references) CNS	Intestine
Histamine		7.4 (2)	6.9 (59) 7.4 (20)
N^{α}-Methylhistamine		7.8 (21)	7.2 (59) 7.9 (72)
N^{α},N^{α}-Dimethylhistamine		7.6 (21)	7.3 (90)
(R)-α-Methylhistamine		8.4 (21)	7.6 (59) 7.8 (23)
Imetit		9.3 (20) 9.0 (22)	8.2 (72)*
Immepip		NT	7.8* 8.0 (23)
α(R),β(S)-Dimethylhistamine		8.5 (89)	NT†

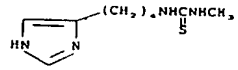
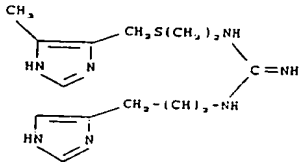
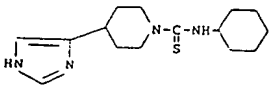
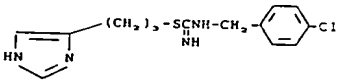
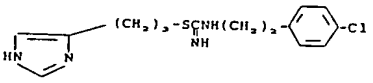
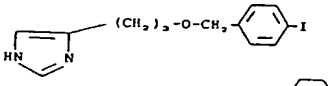
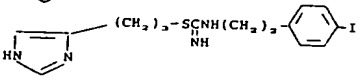
*Poli (unpublished data).
†NT = not tested.

endogenous regulation of stimulated acid secretion. Conversely, the acid secretion induced by direct stimuli (histamine and dimaprit) was not affected by either the agonist or the antagonist. Roughly similar results were obtained in the conscious dog with gastric fistula (38, 39). So far these last studies represent the most exhaustive investigation in which an attempt was made to clarify the mechanism of action of (R)-α-methylhistamine. Together with acid secretion, plasma gastrin levels were also considered: it was of interest that the effect of bombesin and 2-DG on acid secretion was markedly inhibited by (R)-α-methylhistamine, whereas the increase in plasma gastrin levels evoked by the above stimuli was not affected. This was taken as an indication that histamine H_3 receptors do not occur in the G cells, at least in the dog (Figure 1). Preliminary data obtained in the cat (40, Coruzzi, unpublished results) seem to confirm these findings. The hypothesis that H_3 receptors are located in ECL cells exerting an inhibition of histamine release induced by gastrin and/or acetylcholine was supported by very recent observations (Soldani et al, unpublished results) in anesthetized dogs: histamine levels in the gastrosplenic vein increased after pentagastrin, and this increase was strongly reduced (approximately 78%) by administration of (R)-α-methylhistamine. Furthermore, in the same experimental conditions, the histamine release evoked by low doses of pentagastrin was strongly enhanced by thioperamide; this indicates that endogenous histamine may regulate its own release through a negative feedback mechanism involving H_3 receptors. This observation is in full agreement with the early data obtained in the brain (7). Our experiments, however, also support the suggestion that H_3 receptors are located on the cholinergic neurons (Figure 1).

Surprisingly, in most of the experimental models employing the rat, both (R)-α-methylhistamine and thioperamide were completely ineffective (41), with the exception of an inhibitory action of (R)-α-methylhistamine when administered (icv = intracerebroventricularly) in conscious pylorus-ligated rats (42).

A synopsis of the *in vivo* data concerning the effect of (R)-α-methylhistamine in different species is shown in Table 3.

TABLE 3. ANTAGONISTIC ACTIVITY OF SOME H_3 LIGANDS DETERMINED ON RAT CEREBRAL CORTEX AND GUINEA PIG INTESTINE

Compound	Structure	pA_2 values (reference)	
		CNS	Intestine
Burimamide		7.5 (2)	*
Impromidine		7.5 (2)	7.59 (54)
Thioperamide		8.9 (21)	8.79 (72) 7.9 (59)
Clobenpropit		9.5 (26)	9.9 (24)
Clozapropit		NT†	9.7‡
Iodoxyfan		8.3 (35)	9.0 (35)
Iodophenpropit		1.2 nM§	9.6 (90)

*Insurmountable antagonism (Poli, unpublished data).
†NT = not tested.
‡Poli, unpublished data.
§K_D determined in displacement experiments.

In Vitro Experiments

Results obtained by the use of *in vitro* techniques were not as homogeneous as those above described. It is evident from Table 4 that results varied markedly according to the different species and experimental models: in the mouse whole stomach, for instance, (*R*)-α-methylhistamine actually increased acid secretion (43), whereas it had no effect in isolated gastric glands (44). In the rat and the guinea pig (41, Coruzzi, unpublished data), the H_3 agonists and antagonists were ineffective on both basal and stimulated acid secretion. Finally, in rabbit gastric glands (*R*)-α-methylhistamine did not affect acid secretion, whereas thioperamide increased it (45). On the whole, the above results indicate that the commonly used isolated gastric preparations do not seem suitable for the study of H_3 receptors, although they proved suitable for the study of H_2 receptors. They also suggest that histamine H_3 receptors in the gastric mucosa are more likely located in the gastric environment rather than on parietal cells. Several studies of the effect of (*R*)-α-methylhistamine on acid secretion and on hormonal and paracrine mediators confirm the above hypothesis. In fact, besides the above-described inhibitory effect of (*R*)-α-methylhistamine on histamine release induced by pentagastrin observed in the dog, an inhibition of gastrin-induced histamine release by the H_3 agonists was observed *in vitro* in the rat (46). Recently, an exhaustive study carried out by Prinz et al (47) showed that in the rat (*R*)-α-methylhistamine inhibited, whereas thioperamide stimulated, gastrin/CCK8-induced histamine release from the ECL cells. This supports a role for the ECL cells in the peripheral control of gastric acid secretion by histamine H_3 receptors. The apparent contrast between these data and the lack of inhibitory effect of H_3 receptor activation on acid secretion in the rat might perhaps be explained if we consider that

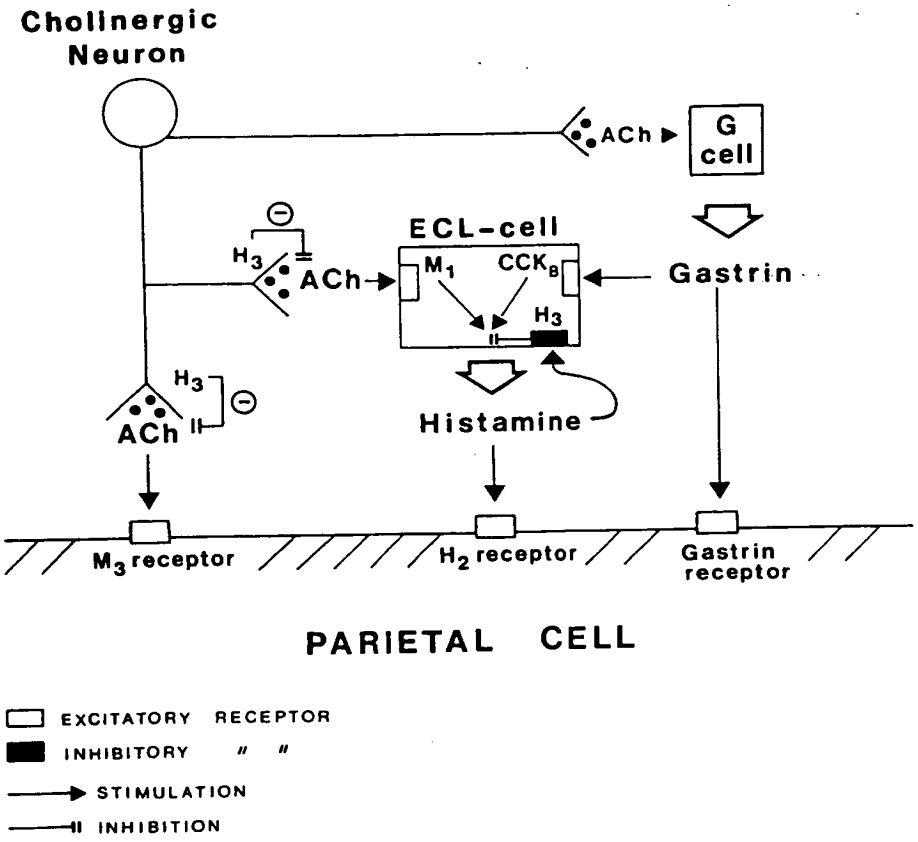


Fig 1. Proposed locations of histamine H_3 receptors in the parietal cell environment, based on experiments performed in conscious dogs. ACh = acetylcholine. (From 39, modified.)

(*R*)-α-methylhistamine, at the concentrations used by Prinz et al (10^{-6} M), may also activate histamine H_2 receptors in the parietal cells, with a consequent increase in acid secretion. This could mask an antisecretory effect due to the decrease of histamine release.

An inhibition of "basal" histamine release from rabbit gastric glands was observed following administration of (*R*)-α-methylhistamine; thioperamide, besides the expected antagonism, *per se* enhanced basal histamine release (45). It is of interest that in the isolated fundic mucosal cells of the rabbit H_3 receptors seem to autoregulate the histamine synthesis through histidine decarboxylase activity, exactly as was described for the first time in the brain (7, 48).

An interesting, although very preliminary, study is that performed on the isolated mouse whole stomach (49). According to the authors, H_3 receptor activation could actually enhance gastrin release, because of an inhibitory action on D cells with a consequent inhibition of somatostatin release. Of course, these findings await confirmation, since only a short abstract has been published so far. In summary, the role of H_3 receptors in the regulation of hormonal and paracrine influences on acid secretion is still to be clarified (Table 5).

GASTROPROTECTION

The role of histamine in gastric mucosal protection against noxious stimuli is unclear, in spite of a number of reports on this topic. However, although some authors consider histamine to be a gastroprotective agent, most reports are in favor of a definite ulcerogenic role of histamine. An explanation for these contrasting data might lie in differences in the experimental models employed. Although a series of H_1- and H_2-receptor agonists and antagonists were tested for their effects on mucosal protection and/or damage, no definite conclusion as regards the subtype of receptors involved was reached from these investigations. The availability of H_3 receptor agonists and

TABLE 3. EFFECTS OF (*R*)-α-METHYLHISTAMINE ON GASTRIC ACID SECRETION (*IN VIVO* DATA)

Species	*Technique*	*Effect**	*Reference*
Conscious cat	Gastric fistula	a) Inhibition of 2-DG- and bombesin-induced secretion b) No effect on dimaprit- and pentagastrin-induced secretion	36
	Gastric fistula and Heidenhain pouch	a) Inhibition of food- and pentagastrin-induced secretion b) No effect on basal and histamine-induced secretion	37
Conscious dog	Gastric fistula	a) Inhibition of 2-DG, pentagastrin and bombesin-induced secretion b) No effect on basal and histamine-induced secretion	38,39
Conscious rat	Pylorus-ligated	No effect after intraperitoneal administration	41
		Inhibition after intracerebroventricular administration	42
Anesthetized rat	Lumen-perfused stomach	No effect on basal, histamine, and 2-DG-induced secretion	41

*2-DG = 2-deoxy-D-glucose.

antagonists seemed to be a good reason to focus on the role of histamine. After a first preliminary observation demonstrating that the selective H_3 receptor agonist (*R*)-α-methylhistamine decreased lesion formation caused by ethanol in the rat (50), a thorough investigation was performed in our laboratory (51, 52) in order to reveal a possible role of H_3 receptors in gastroprotection against a variety of stimuli. From this study it emerged that (*R*)-α-methylhistamine, administered in a dose range of 1–100 mg/kg either by an intragastric or intraperitoneal route, dose dependently inhibited ethanol-induced lesions. The effect induced by 30 mg/kg (*R*)-α-methylhistamine (approximately 50% protection) was completely reversed by thioperamide, suggesting the involvement of H_3 receptors. Conversely, when complete protection was obtained with the H_3 agonist at 100 mg/kg, no antagonism was noted either with thioperamide or with the new and more potent H_3 antagonist, clobenpropit (26). This suggests that at least part of the effect of (*R*)-α-methylhistamine is independent of an activation of H_3 receptors.

TABLE 4. EFFECT OF (*R*)-α-METHYLHISTAMINE ON GASTRIC ACID SECRETION (*IN VITRO* DATA)

Species	*Technique*	*Effect*	*Reference*
Mouse	Whole stomach	Increase in acid secretion	43
	Gastric glands	No effect	44
Rat	Gastric fundus	No effect on basal and histamine-induced secretion	41
	Vascularly-perfused stomach	No effect on basal and gastrin-induced secretion	46
Guinea pig	Gastric fundus	No effect	*
Rabbit	Gastric glands	No effect on basal secretion†	45

*Coruzzi (unpublished data).
†Thioperamide increased basal acid secretion.

TABLE 5. EFFECT OF (*R*)-α-METHYLHISTAMINE ON HORMONAL AND PARACRINE MEDIATORS OF GASTRIC SECRETION PROCESS

Species	*Effect*	*Reference*
Rat	Inhibition of histamine release induced by CCK-8	47
	Inhibition of histamine release induced by gastrin	46
Mouse	Inhibition of somatostatin release	49
	Enhancement of gastrin release	49
Rabbit	Inhibition of histamine release	45
Cat	No effect on gastrin release induced by 2-DG	*
	No effect on gastrin release induced by food	40
Dog	No effect on gastrin release induced by 2-DG and bombesin	38, 39
	Inhibition of histamine release induced by pentagastrin	†

*Coruzzi (unpublished data).
†Soldani (unpublished data).

The mechanism of the protective effect of (*R*)-α-methylhistamine is not entirely clear; the involvement of endogenous prostaglandins seems to be very low, as suggested by the weak effect of indomethacin. However, if we allow that histamine is most probably ulcerogenic, it can be speculated that the activation of H_3 receptors may exert mucosal protection by reducing histamine synthesis and release from mucosal stores. Histological and electron microscopy studies indicate that (*R*)-α-methylhistamine increases the number of mucous granules in surface and neck cells and also promotes a rapid process of reepithelization. Finally, since hemorrhagic necrotic lesions were virtually absent in (*R*)-α-methylhistamine-pretreated rats, an involvement of mucosal microvessels might be hypothesized. It was in fact previously shown (53) that histamine H_3 receptor activation reduces sympathetic tone in the guinea pig gastric microcirculation with consequent increased blood flow. It is of interest that a complete protection against ethanol-induced lesions, such as that induced by (*R*)-α-methylhistamine, has so far been observed only with prostanoids. In preliminary investigations (Morini et al, unpublished results) (*R*)-α-methylhistamine was found to exert a similar, although quantitatively less evident, gastroprotective effect against aspirin + HCl, indomethacin, and stress.

INTESTINAL MOTILITY

The effects of histamine on gastrointestinal motility are mediated by different types of receptors, located either prejunctionally or in the smooth muscle cells (4). While it seems generally accepted that H_1 receptors mediate the excitatory responses of histamine, the characterization of H_2 receptors has been rather complex: the existence of so-called "anomalous" H_2 receptors, sensitive to the agonists but not to the antagonists, has been reported by some authors (18). Moreover, an inhibitory action of histamine on electrically evoked contractions of longitudinal muscle myenteric plexus preparation was reported not to be mimicked by other H_2 agonists and to be blocked only by burimamide (17). The discovery of histamine H_3 receptors in the central nervous system (CNS) has led some investigators to reexamine the effects of histamine on guinea pig small intestine, and it has clearly been shown that the histamine receptor located prejunctionally, which inhibits acetylcholine release in this tissue, had to be characterized as the H_3 receptor (54). Subsequently, many data in the literature demonstrate the occurrence of H_3 receptors in the small and large intestine of the guinea pig, these receptors being located not only on cholinergic neurons but also in noncholinergic nerve endings. This was assessed both in functional studies with electrically stimulated preparations or isolated myenteric neurons (55–61) and in tissues incubated with [^{3}H]choline, in which the release of radiolabeled acetylcholine was measured (62, 63). Table 6 summarizes the inhibitory effects of two H_3-receptor agonists, namely N^{α}-methylhistamine and the more selective compound (*R*)-α-methylhistamine on the release of acetylcholine or nonadrenergic, noncholinergic (NANC) neurotransmitter from different areas of the guinea pig intestine. It can be seen that the two compounds have approximately the same potency in the different experimental models; however, it was demonstrated that (*R*)-α-methylhistamine has a higher degree of selectivity towards H_3 receptors (59). The inhibitory effect of H_3 agonists was always counteracted by the selective H_3 receptor antagonist thioperamide. The pA_2 values (from 7.99 to 9.0) for this

TABLE 6. INHIBITORY EFFECTS (pD_2 VALUES) OF H_3-RECEPTOR AGONISTS ON PERIPHERAL NERVOUS TISSUES OF GUINEA PIG INTESTINE*

Tissue	*Neurotransmitter*	*N^{α}*-MHA	*(R)*-α-MHA	*Reference*
Duodenum	ACh	7.17	7.76	59
	NANC	NT	8.09	60
Jejunum	NANC	NT	8.18	60
Ileum	ACh	8.77	NT	54
	ACh	NT	7.80	56
	ACh	NT	7.76	72
	NANC	7.70	7.60	61
	NANC	NT	8.10	60
	NANC	8.40	8.30	57
Colonic submucous neurons	ACh	10 μM	NT	68
Colon	NANC	NT	8.27	60
Myenteric ganglia	ACh	8.06	NT	55
Longitudinal muscle myenteric plexus†	ACh	NT	7.68	62
	ACh	NT	(10^{-9}–10^{-6} M)	63

*ACh = acetylcholine; NANC = nonadrenergic, noncholinergic. NT = not tested.
†Radiolabeled acetylcholine was measured.

compound closely agrees with previous findings in the CNS (21), and they were very similar among the preparations tested, indicating a homogeneous population of H_3 receptors in the intestinal tract. Recent experiments performed in our laboratory with newly synthesized H_3-receptor agonists (imetit and immepip) and antagonists (clobenpropit and clophenpropit) seem to confirm the above data (Poli et al, unpublished results).

Recently, histamine H_3 receptors were found to occur also in porcine small intestine, where they negatively regulate the release of 5-hydroxytryptamine (64). According to the authors these receptors may be localized directly at the enterochromaffin cells, because the inhibitory effect of *(R)*-α-methylhistamine was not modified by tetrodotoxin, which blocks the neuronal input to the enterochromaffin cells.

Very few reports considered the role of H_3 receptors in the gastrointestinal functions of other species: in an extensive investigation carried out in conscious rats (65) with agonists and antagonists of either subtype of histamine receptor, it was found that H_3 receptors contribute only to the central regulation of intestinal motility by histamine. The MMC-inducing effect elicited by icv histamine was mimicked only by icv *(R)*-α-methylhistamine and not by H_1- or H_2-receptor agonists; however, the paradoxical stimulatory effects on motility observed with thioperamide (either intraperitoneally or icv) indicate that further experiments with a series of selective H_3 agonists and antagonists are needed to clarify the role of H_3 receptors in the regulation of gastrointestinal motility. On the other hand, rat intestine was found to be totally unresponsive to H_3 ligands in *in vitro* experiments (Poli, unpublished results).

Finally, H_3 receptor activation seems to have little influence on mouse gastrointestinal motility *in vivo* (66). In fact *(R)*-α-methylhistamine had no effect on the intestinal transit of a charcoal meal and the inhibitory effect induced by the nonselective histamine receptor agonist, *N^{α}*-methylhistamine, was due to activation of H_1 receptors.

Very few papers considered the effects of histamine H_3 receptor activation on ion fluxes in intestinal mucosa. A recent study concerning histamine receptors on the canine proximal colonic mucosa emphasizes that only H_1 and H_2 receptors are involved in the transport of Cl^- ions, whereas apparently H_3 receptors are functionally absent (67). In contrast, in the guinea pig distal colon it was reported that histamine and dimaprit increased neurally evoked chloride ion secretion, this effect being antagonized by the H_3 receptor agonists *N^{α}*-methylhistamine and *(R)*-α-methylhistamine and enhanced by burimamide and thioperamide (68). This study also suggests the occurrence of H_3 receptors in the cholinergic neurons of the submucous plexus and not in the colonic epithelial cells, taking into account that when neural transmission was blocked, the epithelial responses to secretagogues like VIP and carbachol were not modified by H_3 receptor agonists.

POSSIBLE HETEROGENEITY IN H_3 RECEPTOR POPULATION

In spite of the "principle of receptor parsimony", ie, a new receptor subtype should not be invoked until absolutely necessary (69), several authors have already described the existence of different subtypes of H_3 receptors. An early paper on the topic was that of West et al (70), who showed that in rat brain homogenates [^{3}H]N^{α}-methylhistamine was displaced biphasically by thioperamide and burimamide, and they termed the high-affinity site as "H_{3A}" and the low-affinity site "H_{3B}." Further studies also considered the relative activity of a series of H_3 receptor antagonists. Results so far obtained indicated that mouse and rat brain cortex contain the H_{3A} subtype (71, 72). The same may be said for the guinea pig ileum (72), even though other authors do not agree with this assumption (73). On the other hand in the guinea pig sympathetic postganglionic nerve terminals, histamine receptors seem to belong to the H_{3B} subtype (74). Finally a subpopulation that seems to be actually different from H_{3A} and H_{3B} has been detected in porcine enterochromaffin cells. The above distinction was based on the different activity of thioperamide, burimamide, and impromidine (75). It is obvious that, before accepting the above subclassification, the availability of really selective antagonists of each subtype would be necessary. Moreover, the influence of different experimental models, different species, and kinetic factors should also be considered.

SIGNAL-TRANSDUCING MECHANISMS COUPLED TO HISTAMINE H_3 RECEPTORS

Relatively little information is available regarding the molecular events following activation of H_3 receptors. The inhibition of adenylate cyclase seems to be unlikely, as suggested by experiments in the CNS (76), guinea pig duodenum (77), human fundic glands, and human gastric tumoral cell line HGT-1 (78). In this last model H_3 receptors were reported to be negatively coupled with phosphatidylinositol metabolism (79); however, this was not confirmed in the guinea pig ileum (63). Like many other prejunctional inhibitory receptors, histamine H_3 receptors could be directly coupled, via a G protein, to Ca^{2+} and/or K^+ channels (80). Previous observations in the rat CNS showed that the effects mediated by H_3 receptors are inversely related to extracellular Ca^{2+} concentration and to the frequency of stimulation (81, 82); both these findings are compatible with the concept that H_3 receptors may decrease the intraneuronal availability of Ca^{2+} ions. Indeed, an extensive investigation performed in our lab in the guinea pig duodenum (83) indicates that H_3 receptor-mediated inhibition of acetylcholine release is enhanced in low Ca^{2+} medium and markedly reduced in high Ca^{2+} medium or when intracellular Ca^{2+} concentration is increased by compound Bay K 8644. Thus H_3-mediated effects seem to be associated with a restriction of Ca^{2+} ion access into the nerve terminals; moreover, experiments with ω-conotoxin indicate that neuronal N-type Ca^{2+} channels are involved (Figure 2). In fact the L-type Ca^{2+} channel antagonist nifedipine did not modify the inhibitory effect of (R)-α-methylhistamine. The same mechanism has been reported to explain H_3-mediated inhibition of noradrenaline release in the guinea pig heart (84) and in the mouse brain (85). An alternative hypothesis might be that H_3 receptors are primarily coupled with hyperpolarizing K^+ channels, with consequent reduction in Ca^{2+} entry into the nerve terminal. However, the effect of (R)-α-methylhistamine was not modified in low K^+ medium; in this experimental condition, which enhances K^+ currents, the effects of receptor systems positively coupled with K^+ channels should be enhanced. Moreover, the effect of the H_3 agonist was already blocked by micromolar concentrations of the K^+ channel blocker 4-aminopyridine, concentrations which di-

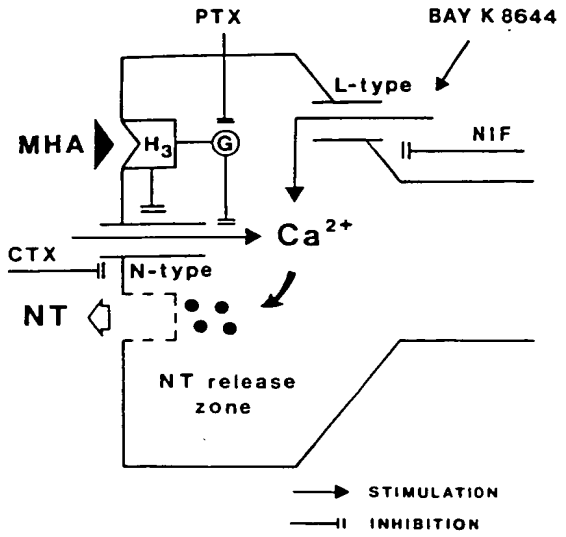


Fig 2. Scheme illustrating the regulation of neurotransmitter (NT) release through histamine H_3 receptors in the intestine. The activation of this receptor by (R)-α-methylhistamine (MHA) can reduce the voltage-dependent Ca^{2+} influx through N-type Ca^{2+} channels. A pertussis toxin (PTX) -sensitive guanine nucleotide regulatory binding (G) protein can be involved in the transduction mechanism. CTX = ω-conotoxin (selective N-type channel blocker); NIF = nifedipine (selective L-type channel blocker).

rectly activate Ca^{2+} channels. Thus a primary activation of K^+ channels following H_3 receptor activation seems to be unlikely (83, 85).

Several papers reporting both binding and functional data support the concept that the signal transduction at H_3 receptors is mediated by a GTP regulatory binding protein (77, 79, 81, 84, 86). This was based on the inhibitory effect of the pertussis toxin and on the regulation of [^{3}H](R)-α-methylhistamine binding by GTP analogues. Thus the H_3 receptor seems to belong, as do the H_1 and H_2 receptors, to the superfamily of receptors with seven transmembrane domains and coupled to G proteins. However, in contrast to the other histamine receptor subtypes that have been recently cloned (87, 88), the molecular structure of the histamine H_3 receptor is still to be elucidated.

GENERAL CONCLUSIONS AND PERSPECTIVES FOR THE FUTURE

In the recent years a growing body of evidence has accumulated that histamine has multiple localizations and multiple roles, apart from the one played in the acid secretion process. The discovery of histamine H_3 receptors and the availability of selective agonists and antagonists clearly reemphasize the role of histamine in all gastrointestinal functions. So far the major role of this new receptor subtype seems to be that of a "braking" mechanism, which operates when gastrointestinal functions are overstimulated by histamine or other mediators released in physiopathological conditions. A better understanding of the function of H_3 receptors could improve the knowledge of the role of histamine as both neuro- and immunomodulator in the gut. Furthermore, more and more selective H_3 ligands could represent a novel class of therapeutic agents for the treatment of deranged gastrointestinal functions.

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Anti-Inflammatory and Antinociceptive Properties of BP 2-94, a Histamine H_3-Receptor Agonist Prodrug

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ABSTRACT

BP 2-94 is an azomethine prodrug of (*R*)-α-methylhistamine [(*R*)-α-MeHA], a potent and selective histamine H_3-receptor agonist. When administered orally to mice BP 2-94 was distributed to various peripheral tissues where it released the active drug. BP 2-94 displayed anti-inflammatory and antinociceptive properties in mice. It dose-dependently inhibited carrageenan-induced paw edema with an ED_{50} value of 0.17 ± 0.05 μmol/kg (p.o.) and a maximal effect of 47%. It also reduced Freund's complete adjuvant-induced paw edema in preventive as well as in curative fashion. Repeated oral administrations of BP 2-94 reduced the pre-established Freund's complete adjuvant-induced edema with an ED_{50} value of 5 ± 2 μmol/kg (p.o.) and a maximal effect of 47%. The antiedema effects of BP 2-94 and indomethacin were additive. BP 2-94 was also efficient in reducing cyclophosphamide-induced cystitis in mice: it decreased leukocyte infiltration by 62% and plasma protein extravasation by 73% in urinary bladder. In addition, BP 2-94 displayed antinociceptive activity in the capsaicin-induced licking test via H_3-receptor stimulation. Its antinociceptive effect was dose dependent, occurring with an ED_{50} value of 0.4 ± 0.1 μmol/kg (p.o.) and a maximal reduction of licking duration by 69%. No tolerance to the antinociceptive effect was observed after repeated administration of BP 2-94 for 3 days. These observations with BP 2-94 suggest that H_3-receptor agonists might represent a novel class of anti-inflammatory and antinociceptive agents.

Histamine (HA) mediates its action via three distinct molecularly and/or pharmacologically well-defined receptor subtypes H_1, H_2, and H_3 (for review, see Schwartz et al., 1991, 1995; Hill et al., 1997). The H_3 receptor has been characterized in brain as a widely distributed prejunctional autoreceptor inhibiting the synthesis and/or release of HA itself (Arrang et al., 1983) as well as of neurotransmitters in adrenergic, cholinergic, nonadrenergic noncholinergic, dopaminergic, and serotoninergic fibers (for review, see Schlicker et al., 1994). The H_3 receptor also plays an inhibition modulatory role in peripheral neurotransmission. Its stimulation inhibits vagal cholinergic transmission in the ileum (Trzeciakowski, 1987; Hew et al., 1990) and the airways (for review, see Barnes, 1992) and reduces plasma protein extravasation induced by sensory C fibers stimulated either electrically or by capsaicin (for review, see Leurs et al., 1998). In addition, H_3 receptor-mediated inhibitions of gastric acid secretion induced either by gastrin or vagal stimulation (Bertaccini and Coruzzi, 1995; Soldani et al., 1996), inhibitions of gastric mucosal injury induced by nonsteroidal anti-inflammatory drugs, cold/restraint stress or ethanol (Belcheva et al., 1997, Morini et al., 1996, 1997), and indirect inhibition of mast cell activity (Dimitriadou et al., 1994, 1997) were reported.

Recently the prototypical agent (*R*)-α-methylhistamine [(*R*)-α-MeHA], a selective and potent H_3-receptor agonist (Arrang et al., 1987), was shown to be extensively methylated by histamine *N*-methyltransferase, rapidly leading to an inactive metabolite (Rouleau et al., 1997). This inactivation process is particularly important in human in which higher hepatic histamine-*N*-methyltransferase activity was detected compared with rat (Brown et al., 1959; Hesterberg et al., 1984). The design of BP 2-94, an azomethine prodrug of (*R*)-α-MeHA (Krause et al., 1995), allowed to minimize the methylation of the imidazole ring of the agonist and thereby markedly improve its oral bioavailability and kinetics in human (Rouleau et al., 1997).

In rodents BP 2-94 was shown to inhibit plasma protein extravasation induced by capsaicin in a large variety of tissues, to display antinociceptive effects in the phenylbenzoquinone-induced writhing or formalin tests, and to reduce zymosan-induced paw swelling (Rouleau et al., 1997). In addition BP 2-94 reduced gastric mucosal lesions induced by ethanol, aspirin, indomethacin, or stress (Morini et al., 1996, 1997; Belcheva et al., 1997). The major aim of the present work was to further explore the anti-inflammatory and an-

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ABBREVIATIONS: HA, histamine; (*R*)-α-MeHA, (*R*)-α-methylhistamine; FCA, Freund's complete adjuvant; HTAB, hexadecyltrimethylammonium bromide; MPO, myeloperoxidase; NK, neurokinin.

tinociceptive profile of the H_3-receptor agonist by using a larger variety of tests in mice.

Materials and Methods

Animals. Male Swiss mice (25–30 g; Iffa-Credo, L'Arbresle, France) were used for all experiments. Food and water were given ad libitum.

Distribution of BP 2-94 and (*R*)-α-MeHA in Mouse Tissues after Oral Administration of BP 2-94. Mice received BP 2-94 (24 μmol/kg) or its vehicle orally. They were sacrificed by decapitation at various times, blood was collected, centrifuged (15,000*g* for 1 min), and the supernatant was brought up to a final concentration of 0.4 N $HClO_4$. Cerebral cortex, lung, liver, and kidneys were dissected out rapidly and homogenized in 10 volumes (w/v) of ice-cold 0.4 N $HClO_4$. Plasma and tissue extracts were then centrifuged, and the clear supernatant was used for assay immediately or stored at −20°C. BP 2-94 and (*R*)-α-MeHA levels were measured by radioimmunoassay as described (Rouleau et al., 1997). Briefly, before use one aliquot of the $HClO_4$ extracts was heated at 95°C for 30 min to allow total in vitro hydrolysis of the prodrug, and another one was used without heating. (*R*)-α-MeHA was derivatized and then radioimmunoassayed in the nonheated and heated extracts. The level of BP 2-94 was calculated as the difference between these two determinations. The plasma and tissues of nontreated mice also were assayed to estimate the interference of plasma and tissues in the RIA for (*R*)-α-MeHA. The determinations of (*R*)-α-MeHA for treated mice were then corrected accordingly.

Freund's Complete Adjuvant (FCA)-Induced Paw Edema. Inflammation of one hind paw of mice was induced by intraplantar injection under ether anesthesia of 10 μl of modified FCA, containing 0.1% heat-sacrificed and dried *Mycobacterium butyricum* in 85% Drakeol 5 NF and 15% Arlacel A (Stein et al., 1988). Control animals were anesthetized but not injected. Mice were sacrificed at various times after the injection of the inflammatory agent, both hind paws were cut off at the ankle and the difference between their weights, representing paw swelling, was calculated. Each hind paw was then put into 4 ml of 50 mM potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HTAB), and homogenized with a Polytron. Homogenates were stored at −20°C until myeloperoxidase (MPO) activity assay. The preventive effect of an acute administration of BP 2-94 was first studied. BP 2-94 (16.4 μmol/kg) or its vehicle was orally administered 1 h before FCA injection, and the time course of its anti-inflammatory effect was determined during the next 16 h. The effect of BP 2-94 (16.4 μmol/kg) also was studied in the presence of indomethacin administered orally at increasing dosages (0.6–28 μmol/kg) (Chan et al., 1995). Next, the curative effect of repeated administrations of BP 2-94 was evaluated. Mice were injected with FCA, and 16 to 18 h thereafter, they received orally BP 2-94 or its vehicle b.i.d. during a period varying from 1 to 9 days. The effect of 3-day repeated administrations of BP 2-94 (16.4 μmol/kg p.o., b.i.d.) was then compared with the effect of 3-day repeated administrations of indomethacin (8.4 μmol/kg p.o., b.i.d.), dexamethasone (7.6 μmol/kg p.o., b.i.d.) (Gegout et al., 1995), or RP67580 (0.7 μmol/kg i.v., b.i.d.), a neurokinin $(NK)_1$-receptor antagonist (Garret et al., 1991). The effect of BP 2-94 (3-day repeated administrations) in association with indomethacin in increasing doses (0.8–28 μmol/kg p.o., b.i.d.) also was studied (Gans et al., 1990).

MPO Activity. Changes in MPO activity represent a reliable index of polymorphonuclear leukocyte infiltration in the inflamed paw (Bradley et al., 1982). Therefore, paw homogenates were freeze thawed three times and centrifuged to collect the supernatant that was used for MPO activity assay adapted to a 96-well plate format (Rao et al., 1994). Briefly, 10 μl of unknowns or human neutrophil MPO standards were added to a 96-well plate. The reaction was initiated by the addition of 200 μl of assay buffer containing 0.167 mg/ml *o*-dianisidine and 0.0005% hydrogen peroxide and absorption measured at 405 nm (Spectrophotometer Dynatech, MR5000). Results are expressed as the difference in MPO activity between the two hind paws.

Carrageenan-Induced Paw Edema. Paw swelling was elicited with 25 μl of 0.5% lambda carrageenan suspension in saline into the right hind paw (Drelon et al., 1994). The left hind paw was injected with 25 μl of saline. BP 2-94 at increasing doses and indomethacin (28 μmol/kg) were administered 1 h before the injection of the carrageenan suspension. Paw edema and MPO activity were measured as described above, 6 h after the induction of the inflammation.

Cyclophosphamide-Induced Cystitis. At various times after cyclophospha-mide injection, plasma protein extravasation and MPO activity were evaluated. For that, one group of mice was anesthetized with pentobarbital (10 mg/kg i.p.), injected with Evans blue dye (30 mg/kg i.v.), and sacrificed 30 min thereafter. The urinary bladder was dissected out, immersed into 0.4 ml of formamide, and maintained at 45°C for 18 h. The extracted dye concentration was measured by spectrophotometry at 630 nm (Dynex, MRX). Another group of mice was sacrificed and the urinary bladder was dissected out and homogenized in 0.4 ml of phosphate buffer (50 mM, pH 6) containing 0.5% HTAB and the resulting homogenates were frozen until MPO assay. Changes in MPO activity also were evaluated 5 h after cyclophosphamide was administered in increasing doses. The H_3-agonist prodrug BP 2-94 (33 μmol/kg p.o.) or its vehicle was administered to mice 1 h before cyclophosphamide and blue Evans content and MPO activity were evaluated 3 and 5 h later, respectively.

Capsaicin-Induced Licking. The capsaicin test was performed according to Sakurada et al. (1992). BP 2-94 or vehicle was given orally 1 h before capsaicin injection. After 30 min, mice were placed individually in transparent cages (26 × 16 × 14.5 cm) that served as the observation chambers. After a 30-min adaptation period, mice received a 20 μl-injection of capsaicin (1.5 μg in saline with 7.5% dimethyl sulfoxide) under the skin of the dorsal surface of the right hind paw. When required, ciproxifan (37 μmol/kg) (Ligneau et al., 1998) was orally administered 1 h before BP 2-94 (1 μmol/kg). The observation period started immediately after the capsaicin injection and lasted for 5 min. The time the animals spent licking the injected paw was evaluated by using a stopwatch.

Analysis of Data. For the determination of ED_{50} (dose responsible for 50% of the maximal effect) and maximal effect, inhibitory effects of drugs were analyzed by using an iterative computer least-squares method derived from that of Parker and Waud (1971), with the following nonlinear regression:

$$\text{Inhibitory effect of the drug} = \frac{[\text{maximal inhibitory effect of the drug}] \times [\text{drug dose}]}{[\text{drug dose}] + ED_{50}}$$

Statistical analyses were by one-way ANOVA followed by Student-Newman-Keuls or Tukey-Kramer's post test.

Drugs and Drug Solutions. BP 2-94 and ciproxifan were from Laboratoire Bioprojet (Paris, France). HTAB, *o*-dianisidine, λ-carrageenan, capsaicin, cyclophosphamide, indomethacin, and dexamethasone were from Sigma Chemical Co. (St. Louis, MO). For oral administration to animals BP 2-94, indomethacin and dexamethasone were dissolved into 1% methylcellulose plus 5% dimethyl sulfoxide. RP67580, a kind gift of C. Garret (Rhône-Poulenc Rorer, Vitry, France), was dissolved in saline. Human neutrophil MPO and FCA were from Calbiochem (La Jolla, CA). All other reagents were from commercial sources and were of the highest purity available.

Results

Distribution of BP 2-94 and (*R*)-α-MeHA in Mouse Tissues after Oral Administration of BP 2-94. After oral administration of BP 2-94 to mice, both the prodrug and the active drug (*R*)-α-MeHA were detected in plasma and various

tissues. Figure 1 shows the fits of concentration versus time profile. The levels of both compounds peaked at 1 h and then declined with a half-life of around 1 h. Similar (*R*)-α-MeHA levels were reached in lung and plasma, whereas levels in liver and kidney were twice as high and hardly detectable in cerebral cortex (data not shown).

Effects of BP 2-94 on FCA-Induced Paw Edema. After preliminary trials a dose of 10 μl FCA was selected and its proinflammatory effects were studied in mice. Swelling of the injected paw was apparent as soon as 1 h after treatment with FCA. The edema increased slightly until 24 h and then slowly subsided, but it was still important at the end of the 9-day observation period (Fig. 2). Changes in MPO activity followed almost the same pattern (data not shown). The FCA-induced paw swelling was reduced by about 50% during at least 16 h in mice receiving BP 2-94 (16.4 μmol/kg p.o.) 1 h before FCA (Fig. 2A). BP 2-94 also reduced paw swelling (by 34–45%) when its chronic administration (16.4 μmol/kg p.o., b.i.d.) started 16 h after the FCA injection, i.e., at a time when the inflammation was firmly established (Fig. 2B). However, BP 2-94 did not affect the FCA-induced increase in MPO activity, whatever its time of administration (data not shown).

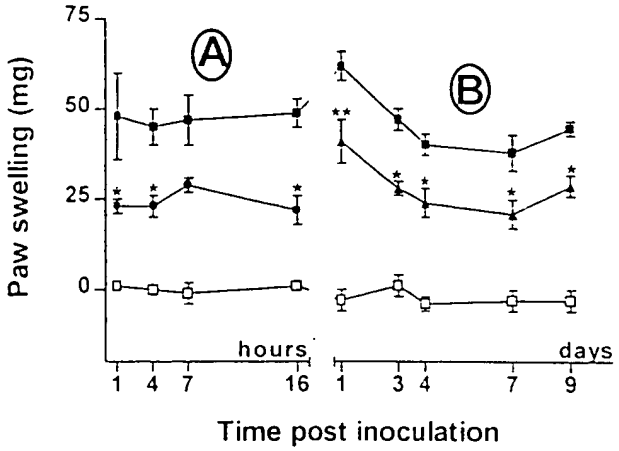

Fig. 2. Anti-inflammatory effect of BP 2-94 on FCA-induced paw edema in mice. A, animals were given vehicle (■) or BP 2-94 (16.4 μmol/kg p.o.) (● or ▲) once 1 h before unilateral intraplantar FCA injection into the hind paw. B, same treatments were given b.i.d. starting from 16 h after FCA. Control animals (□) were anesthetized but not injected. Mice were sacrificed at indicated times after the FCA injection, both hind paws cut off at the ankle, and the difference between their weights calculated. $^*P < .05$, $^{**}P < .01$. Mean ± S.E. of 3 to 10 values.

Repeated administrations of BP 2-94 twice a day for 3 days, starting 16 h after the FCA injection, decreased in a dose-dependent manner the FCA-induced edema with an ED_{50} of 5 ± 2 μmol/kg (b.i.d.) and a maximal effect of 47% (data not shown). The maximal effect of BP 2-94 was close to that of RP67580, whereas indomethacin and dexamethasone in maximal doses elicited a higher anti-inflammatory effect, decreasing paw swelling by 70 and 64%, respectively (Fig. 3, top). Higher doses of RP67580 did not display any higher inhibitory effect (data not shown). In addition, only indomethacin and dexamethasone were efficient in reducing neutrophil infiltration evaluated by the increase in MPO activity (Fig. 3, bottom).

When BP 2-94 was coadministered with indomethacin in increasing doses, either preventively in single administration or curatively in a repeated manner, the anti-inflammatory effects of the two drugs seemed to be additive for nonmaximal doses of indomethacin. However, BP 2-94 (16.4 μmol/kg once or b.i.d. for 3 days) did not increase the maximal effect of indomethacin (28 μmol/kg once or b.i.d. for 3 days) (Fig. 4).

Effects of BP 2-94 on Carrageenan-Induced Paw Edema. After preliminary trials the rat test (Drelon et al.,

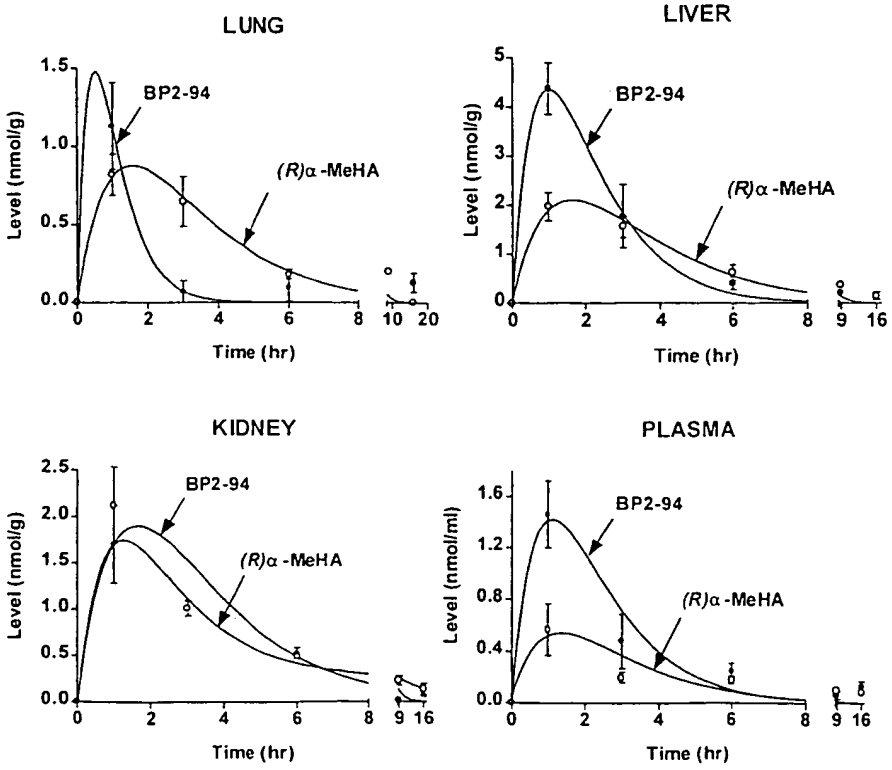

Fig. 1. Tissue and plasma levels of BP 2-94 and (*R*)-α-MeHA immunoreactivities in mice receiving BP 2-94. Groups of four mice were sacrificed at various times after oral administration of BP 2-94 (24 μmol/kg). (*R*)-α-MeHA was radioimmunoassayed in tissue and plasma extracts before and after hydrolysis of BP 2-94 into (*R*)-α-MeHA. BP 2-94 level was calculated as the difference between these two values.

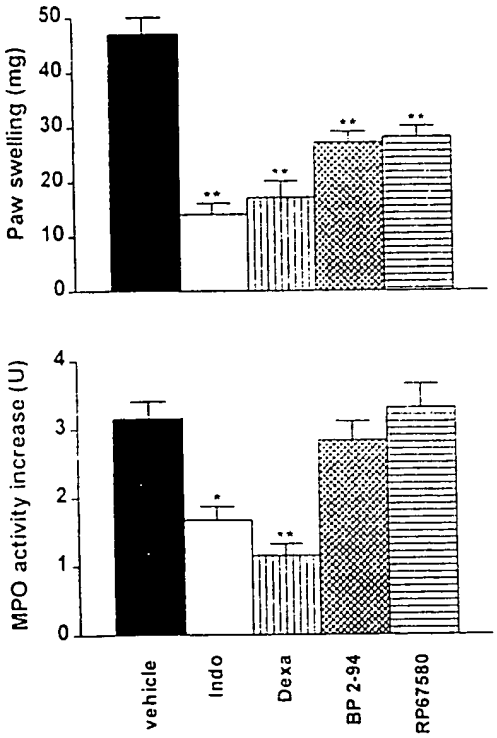

Fig. 3. Effects of repeated administrations of indomethacin, dexamethasone, BP 2-94, and RP67580 on FCA-induced paw inflammation in mice. Mice were given 16 h after unilateral FCA injection, indomethacin (Indo, 8.4 μmol/kg p.o.), dexamethasone (Dexa, 7.6 μmol/kg p.o.), BP 2-94 (16.4 μmol/kg p.o.), RP67580 (0.7 μmol/kg i.v.), or vehicle twice a day. Animals were sacrificed after 3 days of treatment and differences in paw weight (top) and MPO activity (bottom) determined. $^{*}P < .01$; $^{**}P < .001$. Mean ± S.E. of 10 to 20 values.

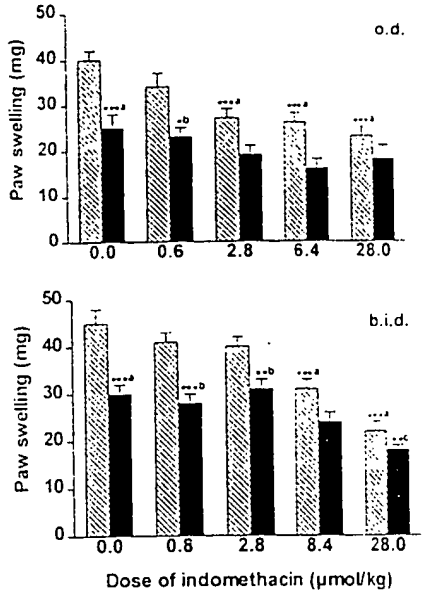

Fig. 4. Additive effects of BP 2-94 and indomethacin on FCA-induced paw edema. Top (single administration), mice were given BP 2-94 (16.4 μmol/kg p.o.) or its vehicle together with increasing doses of indomethacin (0.6–28 μmol/kg p.o.) 1 h before FCA injection and sacrificed 4 h after. Bottom (repeated administration), same treatments as top but b.i.d. for 3 days, starting from 16 to 18 h after FCA injection. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; a, as compared with controls without indomethacin; b, with indomethacin alone (at the same dose); or c, with BP 2-94 alone. Means ± S.E. of 13 to 17 values. ▨, indomethacin; ■, indomethacin + BP 2-94.

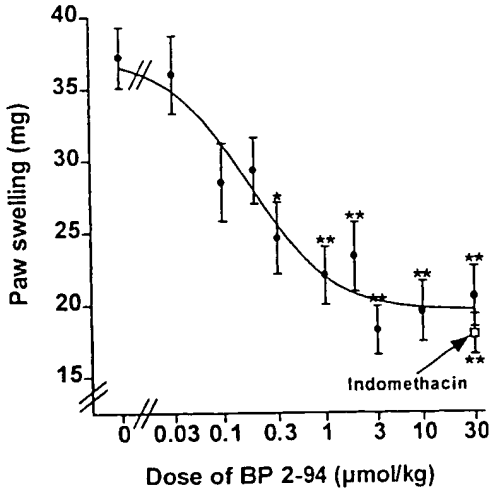

Fig. 5. Effects of BP 2-94 on carrageenan-induced paw edema. BP 2-94 in increasing doses or indomethacin (28 μmol/kg) were administered orally 1 h before the injection of the carrageenan suspension. After 6 h, animals were sacrificed and paw edema was determined. $^{*}P < .01$; $^{**}P < .001$. Mean ± S.E. of 8 to 43 values.

1994) was adapted to mice. Administration of 25 μl of 0.5% carrageenan was selected out and found to induce a moderate inflammation with a maximal edema peaking at 6 h (data not shown). Administered orally 1 h before carrageenan, BP 2-94 decreased in a dose-dependent manner the carrageenan-induced paw swelling measured at 6 h with an ED_{50} of 0.17 ± 0.05 μmol/kg and a maximal reduction of 47%, similar to that elicited by a maximal dose of indomethacin (28 μmol/kg) (Fig. 5), but BP 2-94 had no effect on MPO activity (data not shown).

Effects of BP 2-94 on Cyclophosphamide-Induced Cystitis in Mice. The rat test (Lantéri-Minet et al., 1995) was adapted to mice. Cyclophosphamide (100 mg/kg i.p.) induced plasma protein extravasation in urinary bladder as well as leukocytes infiltration evidenced by an increase in MPO activity, starting 3 h after injection and still present 8 h thereafter. In contrast significant plasma protein extravasation was detected as soon as 1 h after the injection and persisted for at least 5 h thereafter (Ahluwalia et al., 1994) (Fig. 6). From these observations, the times of 3 and 5 h after cyclophosphamide injection were chosen for measurement of plasma protein extravasation and MPO activity, respectively. The effect of cyclophosphamide-induced enhancement of MPO activity was dose related, a maximal effect being obtained with a dose of 200 mg/kg (data not shown). BP 2-94 (33 μmol/kg p.o.) administered 1 h before cyclophosphamide (200 mg/kg) reduced MPO activity by 62% (485 ± 114 versus 186 ± 56 milliunits/bladder for vehicle and BP 2-94-treated mice, respectively) (Fig. 7). The amount of Evans blue dye in urinary bladder was increased by 186% 3 h after cyclophosphamide injection, and pretreatment with BP 2-94 (33 μmol/kg p.o.) reduced this response by 73% (Fig. 7).

Antinociceptive Activity of BP 2-94. The duration of licking induced by capsaicin was significantly reduced in mice receiving BP 2-94 orally. The antinociceptive activity of the compound was dose related and occurred with an ED_{50} of 0.4 ± 0.1 μmol/kg and a maximal reduction of the licking duration by 69% (Fig. 8).

The antinociceptive effect of BP 2-94 (1 μmol/kg) was abolished by previous administration of ciproxifan (37 μmol/kg),

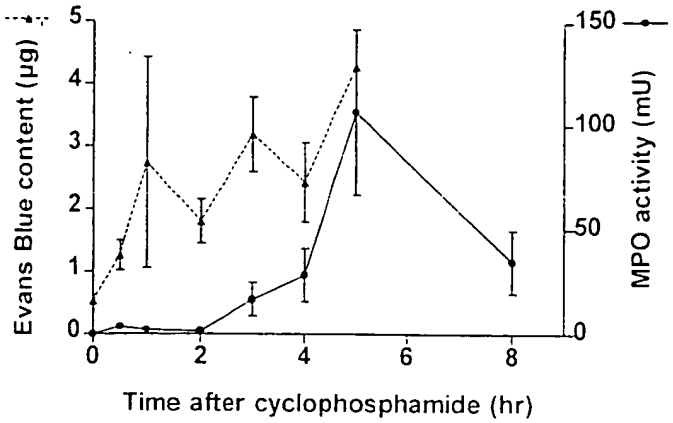

Fig. 6. Time course of cyclophosphamide-induced plasma protein extravasation and MPO activity in urinary bladder. Cyclophosphamide (100 mg/kg i.p.) was injected to groups of mice. One group of animals was anesthetized with pentobarbital (10 mg/kg i.p.), received an injection of Evans blue dye (30 mg/kg i.v.), and was sacrificed 30 min later; the urinary bladder was dissected out and dye extracted and measured. The other group was sacrificed at indicated times, the urinary bladder dissected out, and MPO activity determined.

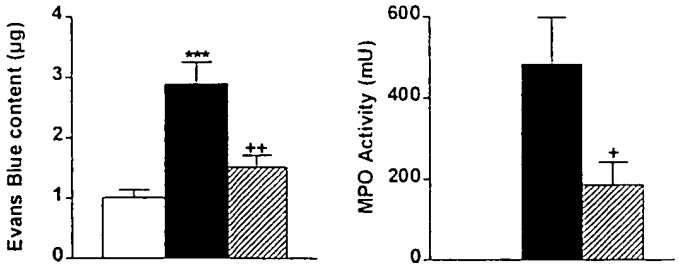

Fig. 7. Effects of BP 2-94 on cyclophosphamide-induced cystitis. Cyclophosphamide (200 mg/kg i.p.) was injected 1 h after BP 2-94 (p.o.) or its vehicle. One group of mice was injected with Evans blue (30 mg/kg i.v.) 2.5 h after cyclophosphamide injection and plasma protein extravasation in urinary bladder was evaluated 30 min later. A second group was sacrificed 5 h after cyclophosphamide injection, the urinary bladder dissected out for MPO activity assay. ***$P < .001$ with control; $^{+}P < .05$; $^{++}P < .01$ with cyclophosphamide. Mean ± S.E. of 9 to 29 values. □, control; ■, cyclophosphamide; ▨, cyclophosphamide + BP 2-94.

a selective H_3 receptor antagonist (Table 1). Ciproxifan alone did not change the duration of licking. BP 2-94 (1 μmol/kg) administered 1 h before capsaicin elicited a similar effect in mice pretreated for 3 days with BP2–94 (1 μmol/kg p.o., b.i.d.) or with its vehicle (Table 1).

Discussion

The present report confirms and extends to several novel tests our initial report that a potent histamine H_3-receptor agonist exerts both anti-inflammatory and antinociceptive effects. The compound studied, BP 2-94, is a prodrug of (*R*)-α-MeHA, which releases it by nonenzymatic hydrolysis (Rouleau et al., 1997). With a radioimmunoassay to measure (*R*)-α-MeHA levels in tissues before and after heat-induced hydrolysis in vitro, both the prodrug and drug levels could be reliably detected in all tissues tested. After oral administration of BP 2-94, levels of both compounds in mouse tissues were generally higher than in plasma, being maximal after 1 h but still detectable after 9 to 16 h. In brain, however, neither the prodrug nor (*R*)-α-MeHA could be detected.

We show herein that administration of the histamine H_3-receptor agonist significantly reduced the paw edema in-

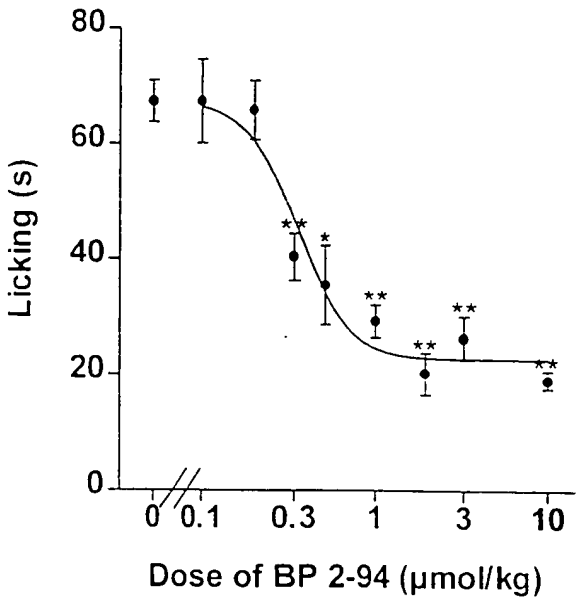

Fig. 8. Antinociceptive effect of BP 2-94 in the capsaicin test. Capsaicin (1.5 μg in 20 μl) was injected under the skin of the dorsal surface of the right hind paw 1 h after BP 2-94 (p.o.) or its vehicle. The duration of licking was measured for 5 min immediately after capsaicin injection. *$P < .01$; **$P < .001$. Means ± S.E. of 4 to 36 values.

TABLE 1

Antinociceptive activity of single or repeated BP 2-94 administration on capsaicin-induced licking

Groups of 8 to 13 mice received BP 2-94 (1 μmol/kg p.o.) 1 h before capsaicin and/or ciproxifan (37 μmol/kg p.o.) 2 h before capsaicin. In B, the effects of repeated treatments with BP 2-94 (1 μmol/kg p.o., b.i.d.) or saline for 3 days before the test were investigated.

Treatment	Licking(s)
A) Saline	71.0 ± 4.6
BP 2-94	26.9 ± 5.1**
Ciproxifan	64.3 ± 8.5
BP 2-94 + ciproxifan	65.5 ± 3.8^{+}
B) Saline	56.8 ± 7.5
BP 2-94 (single administration)	26.7 ± 4.4*
BP 2-94 (repeated administration)	20.2 ± 2.9**

* $P < .01$; ** $P < .001$ compared with saline; $^{+}$ $P < .001$ compared with BP 2-94 alone.

duced by intraplantar administration of either FCA or carrageenan, a similar edema-preventing effect having been previously shown to occur against zymosan (Rouleau et al., 1997). In all three tests, the anti-inflammatory activity of BP 2-94 occurred at low oral dosage (≤1 μmol/kg), and the reduction of FCA-induced edema occurred when BP 2-94 was administered in either a preventive or a curative manner.

Studies of the mediators involved in the edema induced by carageenan have suggested that there are distinct phases in the inflammatory effects of this agent. The first ones, taking place during the first 2 h, involve histamine and kinins as mediators, whereas from 2.5 to 6 h after the injection, prostaglandins seem to be involved (Di Rosa, 1972). The edema-preventing effect of the H_3-receptor agonist was observed during this prostaglandin-mediated phase along which the vascular response and polymorphonuclear cell infiltration reach their maximum. However, the edema-preventing effect of BP 2-94 in the carageenan, zymosan, and FCA tests was not accompanied by any significant attenuation of polymorphonuclear cell infiltration, as assessed by changes in myeloperoxidase activity. In contrast, steroidal and nonsteroidal

inflammatory drugs appear to affect both parameters of inflammation in these three models, as shown herein in the case of the FCA model, whereas in this respect, an NK_1-receptor antagonist displayed a pattern similar to that of BP 2-94 (Fig. 3). Interestingly the maximal-preventing effects of the H_3-receptor agonist on edema were similar to those of indomethacin in the carageenan model, but somewhat lower in the FCA model. In addition, in the latter model the anti-inflammatory effects of the two drugs appeared additive, whatever the dose of indomethacin, consistent with their distinct action mechanisms. Because the anti-inflammatory efficacy of cyclooxygenase inhibitors in either experimental models or in human therapeutics is limited, presumably as a result of the participation of multiple mediators in inflammatory responses, combination of drugs having distinct mechanisms of actions seems a rational approach as long as tolerance is not compromised.

It seems likely that these anti-inflammatory responses to BP 2-94, characterized by a predominant effect upon local edema, are related to the prevention of plasma protein extravasation resulting from the inhibition of tachykinin release. In agreement, stimulation of presynaptic H_3-heteroreceptors on capsaicin-sensitive sensory nerves inhibits tachykinin release and neurogenic plasma leakage in a variety of tissues (Ichinose et al., 1990; Ohkubo et al., 1995; Rouleau et al., 1997).

The marked anti-inflammatory and antinociceptive effects of BP 2-94 on the two other tests used in the present study are also in agreement with such a postulated mechanism. In the cyclophosphamide-induced cystitis, a mouse model that we have adapted from the rat model (Lantéri-Minet et al., 1995), the H_3-receptor agonist significantly attenuated not only plasma protein extravasation but also the increase in myeloperoxidase activity in the inflamed bladder. The existence of a major capsaicin-sensitive component in the plasma protein extravasation induced by acrolein, the active metabolite of cyclophosphamide, was demonstrated, namely, via the use of capsaicin-induced desensitization and an NK_1-receptor antagonist (Ahluwalia et al., 1994). The reasons for which BP 2-94 reduced cyclophosphamide-induced polymorphonuclear influx into bladder, whereas it does not affect this parameter in the inflamed paw in the other models, are not known.

In the capsaicin-induced licking the antinociceptive activity of BP 2-94 can obviously be ascribed to an H_3-receptor-mediated attenuation of primary sensory C-fiber excitation by the pungent principle of hot peppers. Indeed, in this test the nociceptive response is inhibited by intrathecal administration of Substance P antagonists or antibodies (Sakurada et al., 1992). However, the fact that BP 2-94 hardly enters the brain and displays activity in "peripheral" but not "central" tests of nociception suggests that it acts via peripheral H_3-receptors depressing the afferent activity of primary sensory neurons.

All together, the present observations confirm that BP 2-94 exerts clear anti-inflammatory and antinociceptive activities in animal models. As potential anti-inflammatory and analgesic drugs, H_3-receptor agonists theoretically display their advantage over NK_1 (or NK_2) antagonists of reducing the release not only of tachykinins (acting upon various receptor subtypes) but also of other proinflammatory mediators of the sensory fibers such as calcitonin-gene-related peptide, hence a potentially wider therapeutic spectrum. As compared with nonselective cyclooxygenase inhibitors, they display the advantage of lacking ulcerogenic activity and, even more, of displaying gastric mucosa protective ability (Morini et al., 1996, 1997; Belcheva et al., 1997), but they appear to have a more restricted anti-inflammatory spectrum. Compared with selective cyclooxygenase-2 inhibitors, only the more restricted anti-inflammatory spectrum can be invoked, but additive effects of potential therapeutic interest may be anticipated from the combination of the two classes of drugs.

Acknowledgment

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Bioavailability, Antinociceptive and Antiinflammatory Properties of BP 2-94, a Histamine H_3 Receptor Agonist Prodrug[1]

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ABSTRACT

*(R)*α-Methylhistamine [*(R)*α-MeHA], a potent and selective histamine H_3 receptor agonist *in vitro* and *in vivo* in rodents, was found to display comparatively low plasma level in healthy human volunteers, attributable to an extensive methylation of the drug's imidazole ring by histamine-N-methyltransferase. To limit this inactivation process, BP 2-94, *i.e.*, *(R)*-(-)-2-[[N-[1-(1*H*-imidazol-4-yl)-2-propyl]imino]phenylmethyl] phenol, was selected as a prodrug. A sensitive radioimmunoassay was developed to study the generation of *(R)*α-MeHA slowly released from BP 2-94 *in vitro* and *in vivo* by chemical hydrolysis. In mice after oral administration of BP 2-94 high levels of both prodrug and *(R)*α-MeHA were detected in plasma and various tissues except in the brain. In humans receiving 0.1 mmol BP 2-94 orally, plasma levels of *(R)*α-MeHA-like immunoreactivity decayed with a $t_{1/2}$ more than 24 hr, the area under the curve being two orders of magnitude higher than after oral administration of (R)α-MeHA. BP 2-94 displayed antiinflammatory and antinociceptive properties in rodents, related to the H_3 receptor stimulation. It dose-dependently inhibited capsaicin-induced plasma protein extravasation in many rat tissues with ED_{50}s of 0.6 to 14 μmol/kg p.o., and maximal reductions by 35 to 87%. BP 2-94 also reduced zymosan-induced paw swelling in mice with an ED_{50} of 1 μmol/kg p.o. and showed marked activity in the phenylbenzoquinone-induced writhing (ED_{50} = 0.03 μmol/kg, p.o.) or formalin tests in mice, but not in the hot plate jump test. From its pharmacokinetics and pharmacological profile BP 2-94 appears to be a promising novel therapeutic agent in disorders such as asthma, migraine or a variety of inflammatory diseases and pain associated with these disorders.

The HA H_3 receptor mediates presynaptic inhibition of neurotransmitter release on a variety of neuronal systems in the central and peripheral nervous system (Schwartz *et al.*, 1990a, b, 1991). Initially characterized as an autoreceptor controlling HA synthesis in and release from endings of tuberomammillary neurons (Arrang *et al.*, 1983, 1987) it turned out afterward to mediate heterosynaptic inhibition of release of several aminergic transmitters in brain (Clapham and Kilpatrick, 1992; Schlicker *et al.*, 1988, 1989, 1993).

In addition, H_3 receptor stimulation was shown to inhibit vagal cholinergic transmission in the ileum (Trzeciakowski, 1987; Hew *et al.*, 1990) and the airways (Ichinose and Barnes, 1989a; Ichinose *et al.*, 1989) and to reduce plasma protein extravasation induced in the airways (Ichinose *et al.*, 1990; Barnes, 1992) or meninges (Matsubara *et al.*, 1992) by sensory C-fibers stimulation either electrically or by capsaicin. Again the latter effects result from a presynaptic inhibition, in this case of substance P antidromic release (Ichinose and Barnes, 1989b; Ichinose *et al.*, 1990; Matsubara *et al.*, 1992). In addition, H_3 receptor-mediated inhibitions of gastric acid secretion induced either by gastrin or vagal stimulations (Bado *et al.*, 1991; Coruzzi *et al.*, 1991; Soldani *et al.*, 1993, 1996) and inhibition of mast-cell activity (Dimitriadou *et al.*, 1994, 1997) were identified.

Selective stimulation of the H_3 receptor, which has been instrumental in the discovery of these various responses, was made possible by the identification of *(R)*α-MeHA, a prototypic full agonist, displaying 15-fold higher potency than HA at the H_3 receptor and negligible potency at other receptor subtypes (Arrang *et al.*, 1987). Thereafter, other potent and

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ABBREVIATIONS: *(R)*α-MeHA, *(R)*α-methylhistamine; HA, histamine; t-MeHA, N-tele-methylhistamine; t-*(R)*α-diMeHA, N-tele-*(R)*α-dimethylhistamine; Nα, Nα-diMeHA, Nα, Nα-dimethylhistamine; α, α-diMeHA, α, α-dimethylhistamine; Nα-MeHA, Nα-methylhistamine; 2-MeHA, 2-methylhistamine; 4-MeHA, 4-methylhistamine; 2-OH-BZP, 2-hydroxybenzophenone; HMT, histamine-N-methyltransferase; PQ, phenylbenzoquinone; BZQ, benzoquinone; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; A.U.C., area under the curve; RIA, radioimmunoassay; REA, radioenzymatic assay; ir, immunoreactivity; CGRP, calcitonin-gene-related peptide.

selective H_3 receptor agonists, *i.e.*, (αR, βS)α, β-dimethylhistamine (Lipp *et al.*, 1992), imetit (Garbarg *et al.*, 1992; Howson *et al.*, 1992; van der Goot *et al.*, 1992), imepip (Vollinga *et al.*, 1994) and immepyr (Shih *et al.*, 1995) were designed.

Taking into account some of these actions, it was considered that H_3 receptor agonists display a potential therapeutic value in several fields. *(R)*α-MeHA was the first agent to be introduced in phase I clinical trials during which, however, its low plasma level after oral administration, unexpected from animal studies, was discovered (A. Rouleau, M. Garbarg, J.-M. Lecomte, and J.-C. Schwartz, unpublished observation).

In the present set of studies we have identified important methylation of *(R)*α-MeHA as the cause for this important drawback. Therefore, we designed a series of azomethine prodrugs of *(R)*α-MeHA (Krause *et al.*, 1995) able to release slowly the bioactive compound, thus protecting its early metabolic degradation. Among these prodrugs, we have selected BP 2-94, *i.e.*, *(R)*-(-)-2-[[N-[1-(1*H*-imidazol-4-yl)-2-propyl]imino]phenylmethyl] phenol (fig.1) for further development. We show here that, when administered orally to rodents and human volunteers, this compound is readily absorbed, releases *(R)*α-MeHA in plasma and tissues, and displays anti-inflammatory and antinociceptive properties in rodents.

Methods

Radioenzymatic assay of (R)α-MeHA. Before developing a RIA *(R)*α-MeHA was determined in plasma using a REA developed by Garbarg *et al.* (1989b) based on the observation that *(R)*α-MeHA is a substrate for HMT and its methylated derivative is readily extractible into chloroform (Hough *et al.*, 1981). Briefly, *(R)*α-MeHA was mixed with [^{3}H]S-adenosyl-methionine and a preparation of rat kidney HMT purified according to the method of Bowsher *et al.* (1983) slightly modified by Garbarg *et al.* (1989b). The reaction was stopped after 1 hr incubation at 25°C by addition of perchloric acid and the methylated derivative extracted into chloroform and quantified by liquid scintillation spectrometry.

RIA of (R)α-MeHA and BP 2-94. The immunogen was prepared according to Ternynck and Avrameas (1977). *(R)*α-MeHA was cross-linked to BSA using BZQ in a two-step reaction as described for HA and t-MeHA (Garbarg *et al.*, 1989a) with slight modifications. Briefly, BSA (10 mg) was treated with 45 mg of BZQ. After 1-hr reaction the BZQ-BSA formed was purified by passing it through a Sephadex G-25 column and then mixed with 10 mg *(R)*α-MeHA for a 24-hr incubation. After dialysis against water using [^{3}H]*(R)*α-MeHA as a tracer in the coupling reaction, it was estimated that approximately 13 molecules of *(R)*α-MeHA were conjugated to each BSA molecule (*i.e.*, 45 μg antigen per mg of BSA). *(R)*α-MeHA-BZQ-BSA conjugate (0.15 mg/animal) was injected i.d. to three female rabbits (New Zealand, Iffa-Credo, France) according to Garbarg *et al.* (1989a). Antibodies with sufficient titer and affinity were obtained after approximately 6 mo.

To obtain the tracer, the dipeptide Leu-Tyr was [^{125}I]iodinated (Hunter and Greenwood, 1962; Garbarg *et al.*, 1989a). Leu-Tyr (0.01 mg in 10 μl of 0.04 M potassium phosphate buffer, pH 7.5) was mixed with [^{125}I]NaI (1 mCi) and a chloramine T solution. After 5 min the reaction was stopped by addition of a sodium metabisulfite solution. [^{125}I]-Leu-Tyr solution was directly coupled to BZQ (40 mg/ml of ethanol) at room temperature in the dark during 1 hr at pH 6. A chloroform extraction was performed, and the aqueous phase was treated with *(R)*α-MeHA (1 mg/kg free base). The mixture was kept overnight at room temperature in the dark and was then purified by HPLC. The derivatized *(R)*α-MeHA-BZQ-Leu-[^{125}I]Tyr was eluted with a retention volume of 10 ml. The iodinated product was diluted with ethanol and kept at -20°C until used.

Samples were derivatized with BZQ. The procedure was performed on 40 μl of 0.4 N perchloric extracts from *(R)*α-MeHA standard solution, plasma or tissues. A BZQ solution (0.6 mg dissolved in 20 μl ethanol) and 12 μl of 2.5 M triethanolamine were added to the extract before a 30-min incubation at room temperature. The excess of BZQ was trapped by addition of 10 μl 2 M glycine, and the volume was adjusted to 0.2 ml with 0.05 M potassium phosphate buffer pH 7.4 containing 0.1% BSA and 0.01% sodium azide.

All reagents of the RIA were diluted in a 0.1% BSA solution made up in 50 mM potassium phosphate buffer, pH 7.4. Standards, plasma and tissue extracts were run in triplicate. Derivatized materials (60 μl) were mixed with 30 μl diluted serum (1:32,000 in the final incubation medium), and the mixture was subsequently preincubated for 5 to 6 hr at room temperature. Then 75 μl of this mixture and 25 μl of the [^{125}I]iodinated tracer (*(R)*α-MeHA-BZQ-Leu-[^{125}I]Tyr, 10 pM) were incubated together overnight at 4°C in a swine antirabbit IgG-coated 96-well plate. Wells were washed with 0.05 M PBS pH 7.4 buffer containing 0.05% Tween 20, and bound radioactivity was counted in a gamma spectrometer with an efficiency of 82%.

The same assay was used to evaluate BP 2-94 after its total hydrolysis into *(R)*α-MeHA by heating samples diluted with perchloric acid (0.4 N final concentration) at 95°C for 30 min.

A comparison of the two methods to measure *(R)*α-MeHA in the plasma from six human volunteers after an oral dose of *(R)*α-MeHA has been performed and similar results were obtained: 5.3 ± 1.1 and 4.8 ± 0.8 ng/ml after RIA and REA, respectively, applied to samples obtained from humans 2 hr after receiving orally 1.4 mmol of (R)α-MeHA.

RIA of N-tele-(R)α-dimethylhistamine. Based on its 100% cross-reactivity with t-MeHA, t-*(R)*α-diMeHA was radioimmunoassayed using antibodies raised against t-MeHA (Garbarg *et al.*, 1989a). Briefly, after treatment of plasma or tissues with perchloric acid (0.4 N final concentration) and derivatization with BZQ, t-*(R)*α-diMeHA was mixed with a [^{125}I]iodinated tracer (t-MeHA-BZQ-Leu-[^{125}I]Tyr) and the antibodies. After 15 to 18 hr at 4°C, the bound radioactivity was precipitated and counted.

BP 2-94 hydrolysis *in vitro*. A 10 mM BP 2-94 solution prepared extemporaneously in DMSO was diluted to a final concentration of 4 μM in 0.4 N perchloric acid, 0.05 M potassium phosphate buffer, pH 7.4, or rat liver homogenates (prepared in 5 volumes, w/v, of 0.05 M Tris buffer pH 7.5) and incubated at 20, 37 or 95°C. At various time intervals, an aliquot was withdrawn, diluted and brought up to a final concentration of 0.4 N perchloric acid. Samples were immediately derivatized with BZQ and then radioimmunoassayed for *(R)*α-MeHA. Hydrolysis was evaluated as the amount of *(R)*α-MeHA formed during incubation. A blank value corresponding to the *(R)*α-MeHA level at zero time incubation (and representing hydrolysis occurring during the RIA) was determined and subtracted.

HMT activity. HMT activity was quantified by measuring the conversion of HA, *(R)*α-MeHA and BP 2-94 into corresponding tritiated derivatives, methylated in N-tele-position of the imidazole ring by using [^{3}H]S-adenosyl-methionine as [^{3}H]methyl donor. HMT was purified from rat kidney according to the method of Bowsher *et al.* (1983) slightly modified by Garbarg *et al.* (1989b). HA, *(R)*α-MeHA or BP 2-94 were incubated at increasing concentrations with HMT and a mixture of unlabeled and [^{3}H]labeled S-adenosylmethionine (5 μM final concentration) for 1 hr at 25°C. The reaction was stopped by addition of perchloric acid (0.4 N final concentration). Tritiated methylated derivatives were extracted into toluene-isoamyl alcohol (3:2) and quantified by liquid scintillation spectrometry.

[^{3}H]HA release from synaptosomes of rat cerebral cortex. Release experiments with synaptosomes were performed according to Garbarg *et al.* (1992). Briefly, a crude synaptosomal fraction from rat cerebral cortex was preincubated for 30 min with [^{3}H]L-histidine (0.4 μM) at 37°C. After extensive washing, synaptosomes were resuspended in fresh 2 mM K^+-Krebs-Ringer's medium and BP 2-94 alone or together with HA (1 μM) was added. After 5 min the

synaptosomes were depolarized by bringing the K^+-concentration to 30 mM for 2 min. Incubations were ended by a rapid centrifugation and [^{3}H]HA levels determined in the supernatant according to Garbarg *et al.* (1983).

Pharmacokinetic studies in healthy human volunteers. The design of these phase I clinical studies was approved by a local Ethical Committee and the studies authorized by the Agence Française du Médicament. They were performed in a specialized Phase I Clinical Study Center.

The subjects were Caucasian males aged 18 to 35 yr of standard weight who were eligible when their cardiovascular, blood and urine parameters were considered as normal after preenrolment examinations. They had not received any drug during the 2 preceding wk, did not suffer from any acute or chronic disease and were not considered as strong tobacco or alcohol consumers. After receiving their informed consent the subjects, having fasted overnight, received the drug (either *(R)*α-MeHA or BP 2-94) at 8.00 in one capsule that was swallowed with 150 ml water. They had a light breakfast at 10.00 and standard meals at 13.00 and 19.00; they had also to drink 150 ml water at 9.00 and 11.00. Blood samples (5 ml) were serially withdrawn via a catheter implanted in an arm vein, received in test tubes containing heparin, which were immediately centrifuged (3000 rpm, 8 min at 4°C). The supernatant plasma was then separated and frozen at -80°C until it was assayed. Cardiovascular, respiratory, blood cell and chemistry parameters were monitored all along the study.

Capsaicin-induced plasma extravasation. Male Wistar rats (100-150 g, Iffa-Credo) were administered *(R)*α-MeHA or BP 2-94 orally in increasing doses or their vehicle. After 90 min they were anesthetized with pentobarbital (6 mg/kg, i.p.). In one group of rats the following tissues were examined: skin (ears), eye conjunctiva, nasal mucosa, trachea, main bronchi, esophagus and urinary bladder (average weights: 548, 24, 56, 25, 25, 46 and 56 mg, respectively); they received capsaicin (90 μg/kg, i.v.) together with Evans blue dye (30 mg/kg) or vehicles 2 hr after oral treatment. Five min after capsaicin treatment animals were perfused with saline via the left cardiac ventricule for 2 min (constant flow: 24 ml/min) to remove intravascular dye. Tissues were dissected out and analyzed for extravasated Evans blue. Where specified trachea and main bronchi were analyzed together and designated "airways." Dye extraction was carried out by the method of Gamse *et al.* (1980). Tissues were immersed in formamide and maintained at 45°C for 18 hr. Extracted dye was measured by its absorption at 630 nm using a spectrophotometer (Dynatech, MR5000). Another group of animals were used to examine extravasation in dura mater (average weight: 17 mg) using a more sensitive method. Rats were administered FITC-labeled bovine serum albumin (FITC-albumin) (50 mg/kg, i.v.) or its vehicle 115 min after BP 2-94 and 5 min before capsaicin (300 μg/kg, i.v.) or its vehicle. Five min later, animals were perfused as described above, dura mater was carefully dissected out and homogenized in saline phosphate buffer. After centrifugation (15,000 g × 1 min) the fluorescence intensity (excitation wavelength 492 nm, emission wavelength 520 nm) (Kurose *et al.*, 1994) was evaluated in the supernatant using a fluospectrometer (Jobin Yvon, JY3D). A control value corresponding to the basal dye level in the absence of capsaicin injection was determined for each tissue and subtracted. The inhibitory effect of H_3 receptor agonists on capsaicin-induced extravasation was calculated for each dose used as the percent ratio of: [dye (or FITC-albumin) concentration after administration of capsaicin plus BP 2-94 (or *(R)*α-MeHA) minus concentration after capsaicin alone] over concentration after capsaicin alone. These calculated values were analyzed with an iterative computer least-squares method derived from that of Parker and Waud (1971) as described below (see Analysis of data) and ED_{50} values and maximal inhibitory effects were deduced.

Antinociceptive activity. Male Swiss mice (20-25 g, Iffa-Credo) were used in three tests. The phenylbenzoquinone-induced writhing test was performed as described by Chaillet *et al.* (1983). BP 2-94, aspirin (Siegmund *et al.*, 1957), *(R)*α-MeHA or vehicle were given orally before injection of phenylbenzoquinone (PQ) (2 mg/kg, i.p.), and 10 min later, the number of writhing episodes evaluated during a 10-min observation period. When required, thioperamide (51 μmol/kg) or naloxone (30 μmol/kg) were administered i.p. 1 hr before BP 2-94 (0.16 μmol/kg, p.o.). The rather high doses of thioperamide and naloxone was selected to ensure H_3 or opiate receptor blockade for over 1 hr (von Voigtlander and Lewis, 1988; Garbarg *et al.*, 1989b; Fujibayashi and Iizuka, 1995). The inhibitory effect of increasing doses of BP 2-94 on PQ-induced writhing was determined as the percent ratio of the difference of writhing score after vehicle and BP 2-94 treatment over score after vehicle alone, and used to calculate the ED_{50} value and maximal effect of BP 2-94 as mentioned below (see "Analysis of data").

The formalin paw test was performed as described (Rupniak *et al.*, 1993). The duration of licking and biting was recorded after s.c. injection of 20 μl formalin in the dorsal surface of mouse hind paw. The early phase response was recorded immediately after 0.2% formalin injection and for 5 min. The late phase response was recorded beginning 20 min after injection of 1% formalin and continued for 10 min. BP 2-94 (16 μmol/kg), morphine (35 μmol/kg) or vehicle were given orally 1 hr before formalin injection.

The hot plate jump *test* was performed as described by Eddy and Leimbach (1953). One hour after oral administration of BP 2-94 (16 μmol/kg), morphine (35 μmol/kg) or vehicle animals were placed into a plexiglass chimney (height: 19 cm, diameter: 13 cm) on a platform maintained at 55°C, and the time elapsed until they jumped from the platform was measured. The cut-off time was 240 sec.

Zymosan-induced edema in mice. Edema was induced in male Swiss mice (20-25 g) by injecting 25 μl of 0.05% zymosan suspension in saline into the left hind paw (Stefanova *et al.*, 1995). The right hind paw was used as a control and was injected with 25 μl of 0.9% saline. The animals were killed 4 hr later, both hind paws were cut off at the ankle, and the difference between their weights was calculated. BP 2-94 was orally administered 1 hr before, simultaneously with or 1 hr after zymosan injection. The effect of BP 2-94 was also studied in the presence of thioperamide (51 μmol/kg) administered i.p.1 hr before BP 2-94.

Analysis of data. For determination of ED_{50} values and maximal effects, inhibitory effects of drugs were analyzed with an iterative computer least-squares method derived from that of Parker and Waud (1971), using the following non linear regression:

$$\text{inhibitory effect of the drug} = \frac{[\text{maximal inhibitory effect of the drug}] \times [\text{drug dose}]}{[\text{drug dose}] + ED_{50}}$$

Half-lives have been determined by a computerized program using the following nonlinear regression: $y = A\exp^{-Bt} + C\exp^{-Dt} + E\exp^{-Ft}$. Statistical analysis were by one-way analysis of variance and Dunnett's or Tukey-Kramer's post tests with a preset probability level of $P < .05$.

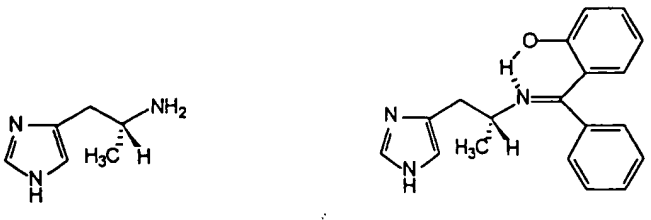
Radiochemicals, drugs and drug solutions. S-adenosyl-L-[^{3}H]methyl methionine (70 Ci/mmol), and [^{125}I]NaI (2,000 Ci/mmol) were from Amersham (Amersham, U.K.). Tritiated *(R)*α-MeHA (10 Ci/mmol) was prepared as described by Arrang *et al.* (1990). t-MeHA was from Sigma Chemical Co. (St. Louis, MO), and t-*(R)*α-diMeHA, α, α-diMeHA were synthesized by one of us (W.S.). Nα-MeHA, 2-MeHA, 4-MeHA and Nα, Nα-diMeHA were from Smith Kline Beecham (London, UK). 2-OH-BZP was from Acros (Pittsburgh, PA). BP 2-94 and *(R)*α-MeHA were from Laboratoire Bioprojet (Paris, France). For administration to animals, BP 2-94, *(R)*α-MeHA or morphine and aspirin (Sigma) were introduced into 1% methylcellulose plus 5% DMSO; naloxone (Sigma) and thioperamide (a kind gift of Pr. Robba, Caen) were solubilized in DMSO, PQ (Sigma) was dissolved in 0.9% NaCl, 3% ethanolic. Capsaicin (Sigma) was solubilized in saline:DMSO:distilled water (36:3:1). Evans blue dye was

prepared in 0.9% NaCl and filtered (Millipore, 0.45-μm pore diameter) before use. FITC-albumin (Sigma) was diluted in 0.9% NaCl. Zymosan (Sigma) was suspended in 0.9% saline by sonication and heated for 30 min at 100°C before use. All chemicals were from Sigma except BZQ (reagent grade) which was from Fluka AG (Buchs, Switzerland), synthetic peptides which were from Bachem (Bubendorf, Switzerland), and S-adenosyl-L-methionine which was from Boehringer (Mannheim, FRG). All other reagents (analytical grade) were from commercial sources and were of the highest purity available.

Results

Characteristics of the RIA for (R)α-MeHA. The RIA was designed using a pool of bleedings exhibiting the best binding parameters. The final antiserum dilution used to obtain 10 to 20% binding of the tracer, in the absence of competing derivatized amine (B_0) was 1:32,000. The pH of the reaction and the BZQ concentration were selected to minimize the interference of BZQ with the antibodies and to obtain the maximal derivatization yield. Addition of *(R)*α-MeHA derivatized with BZQ progressively inhibited the binding of the [^{125}I]tracer to antibodies (fig. 2) with an IC_{50} of 0.5 ± 0.1 nM. The detection limit, defined as the concentration corresponding to 20% inhibition, was 10 pg/well (2 ng/ml). The specificity of the antibodies was tested by measuring the cross-reactivities of various BZQ-derivatized compounds (fig. 2). The cross-reactivity of HA was 7% and that of histidine, 2-OH-BZP and various methylated derivatives of HA was less than 0.05%. The apparent cross-reactivity of BP 2-94 (1-2%) reflects hydrolysis of BP 2-94 into *(R)*α-MeHA taking place during the 30-min derivatization step. Dilutions of derivatized plasma from rats pretreated with *(R)*α-MeHA (0.24 mmol/kg, p.o.) inhibited the binding of the [^{125}I]tracer to antibodies with an inhibition curve paralleling that of the standard (fig. 2). The recovery of *(R)*α-MeHA added to plasma or tissue extracts was about 90%, and all results were corrected accordingly.

In vitro hydrolysis of BP 2-94. The compound BP 2-94 was incubated to estimate its ability to release *(R)*α-MeHA under various conditions. The hydrolysis rates of BP 2-94 at room temperature were similar in neutral and strongly acidic media, representing about 4% per hour. To investigate a possible enzymatic hydrolysis of BP 2-94 incubations were also performed in the presence of rat liver homogenates at 37°C. Under such conditions the hydrolysis rate was in the same range as that obtained in the absence of tissue. Conditions ensuring a complete hydrolysis of BP 2-94 into *(R)*α-MeHA were also investigated for measuring BP 2-94 levels in biological samples. Thus, incubation of BP 2-94 in an acidic medium at 95°C for 30 min, leading to a complete hydrolysis, was selected. The specificity of the assay was assessed in mouse plasma samples having received 0.3 mmol/kg of BP 2-94 p.o. and killed 1 hr later: HPLC analysis on a C_{18} μBondapak column showed only two immunoreactive peaks corresponding to *(R)*α-MeHA and BP 2-94, the latter after hydrolysis (retention times 6 and 28 min, respectively; linear gradient of 10-50% AcN in 10 mM $AcONH_4$ over 30 min).



*(R)*α-Methylhistamine BP 2-94

Fig. 1. Chemical structures of *(R)*α-MeHA and BP 2-94.

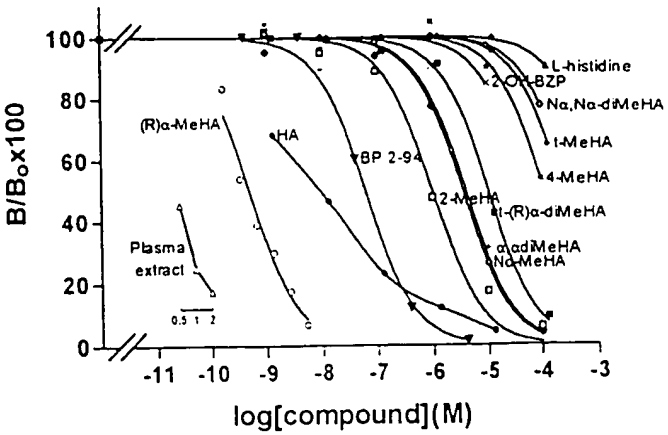


Fig. 2. RIA of *(R)*α-MeHA: Inhibition of [^{125}I]tracer binding to antibodies by various compounds and plasma extracts from rats pretreated with *(R)*α-MeHA. Rats received *(R)*α-MeHA (0.24 mmol/kg, p.o.) 2 hr before being killed and their plasma was radioimmunoassayed for *(R)*α-MeHA after dilution and derivatization with BZQ. All compounds were pretreated with BZQ as described in "Methods," preincubated in the presence of antiserum and incubated with a 10 pM concentration of [^{125}I]tracer.

Methylation of (R)α-MeHA and BP 2-94 by HMT. BP 2-94 was tested as a possible substrate of HMT in comparison with HA and *(R)*α-MeHA. The K_M values of HMT were 3.0 and 2.2 μM and the V_{max} values 2.2 and 1.7 nmol/mg/hr for HA and *(R)*α-MeHA, respectively, but methylation was not detectable with BP 2-94 as a substrate using the standard assay.

Effects of BP 2-94 and t-(R)α-diMeHA on [^{3}H]HA release from rat brain synaptosomes. BP 2-94 (in concentrations from 1 μM to 1 mM) tested as an H_3 receptor antagonist was ineffective in preventing the inhibition of depolarization-induced [^{3}H]HA release from brain synaptosomes elicited by 1 μM HA. When tested as an agonist in concentrations from 1 to 100 μM, BP 2-94 inhibited [^{3}H]HA release by up to 72% with an EC_{50} of about 10 μM to be compared with 4.0 ± 0.9 nM for *(R)*α-MeHA (Garbarg *et al.*, 1992). t-*(R)*α-diMeHA inhibited [^{3}H]HA release with an EC_{50} about 50 μM.

Distribution of BP 2-94 and (R)α-MeHA in mouse tissues after oral administration of BP 2-94. After oral administration of 24 μmol/kg of BP 2-94 to groups of four mice, both the prodrug and the active drug *(R)*α-MeHA-ir were detected in plasma and various tissues as early as 30 min later. The levels of both compounds peaked at 1 hr and then declined with a half-life of about 1 hr. In figure 3 A.U.C.s derived from these data are shown which indicate that similar levels were reached in lung and plasma, whereas levels in liver and kidney were twice as high and hardly detectable in cerebral cortex. C_{max} values (in nmol/g or nmol/ml) were 1.1, 1.7, 4.4 and 1.5 for BP 2-94 in lung, kidney, liver

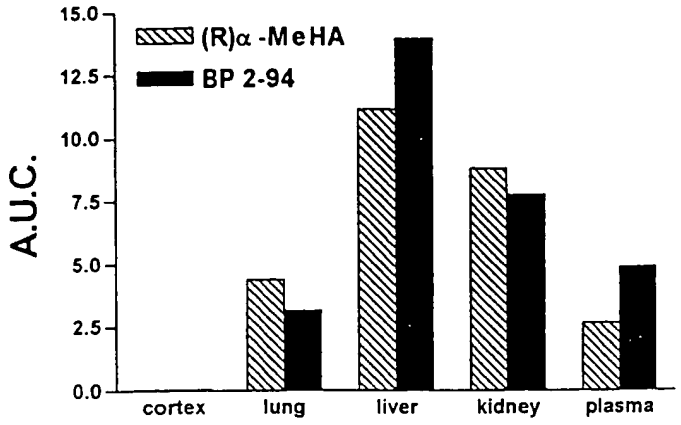


Fig. 3. Tissue and plasma levels of BP 2-94 and *(R)*α-MeHA immunoreactivities in mice receiving only BP 2-94: compared areas under the curves. Groups of four mice were killed at various times (1-16 hr) after oral administration of BP 2-94 (24 μmol/kg). *(R)*α-MeHA was radioimmunoassayed in tissue and plasma extracts before and after hydrolysis of BP 2-94 into *(R)*α-MeHA. BP 2-94 level was calculated as the difference between these two determinations. Results expressed in nmol.hr.g^{-1} for tissues and in nmol.hr.ml^{-1} for plasma.

and plasma, respectively, whereas corresponding values for *(R)*α-MeHA were 0.8, 2.1, 2.0 and 0.6.

Pharmacokinetics of (R)α-MeHA and BP 2-94 in human volunteers. In human volunteers receiving 1.4 mmol of *(R)*α-MeHA orally the plasma level of *(R)*α-MeHA (determined by a REA) was maximal 1.7 hr after the administration and decayed with an apparent half-life of about 1.6 hr. The levels of t-*(R)*α-diMeHA immunoreactivity displayed similar changes. C_{max} values were 40.2 pmol/ml and 3.0 nmol/ml for *(R)*α-MeHA and t-*(R)*α-diMeHA, respectively. In this study, the A.U.C. of plasma t-*(R)*α-diMeHA levels represented 141-fold that of the plasma *(R)*α-MeHA level (table 1). For the purpose of comparison, plasma levels of *(R)*α-MeHA and its methylated derivative were also evaluated in mice receiving 24 μmol/kg of *(R)*α-MeHA orally and A.U.C.s calculated (table 1). In contrast to the data in human volunteers the ratio of the two A.U.C.s was only about 1.5.

In human volunteers receiving 0.1 mmol of BP 2-94 orally the plasma levels of BP 2-94 and *(R)*α-MeHA (both determined by RIA) reached a plateau at 1 to 2 hr and then decayed very slowly in a biphasic manner with similar half-lives, *i.e.*, $t_{1/2}$(a) of about 1 hr and $t_{1/2}$(b) > 24 hr (fig. 4A). After 24 hr, levels of *(R)*α-MeHA-ir and BP 2-94 were still detectable. The A.U.C. of BP 2-94 was 8.0 nmol.hr.ml^{-1} and represented 10-fold that of *(R)*α-MeHA. A similar ratio was obtained for the C_{max} values of BP 2-94 and *(R)*α-MeHA. For

TABLE 1

Plasma levels of (R)α-MeHA and its methylated metabolite after oral administration of (R)α-MeHA to mice and healthy human volunteers

	A.U.C. (pmol · hr · ml^{-1})	
	Mice	Humans
(R)α-MeHA	1,435	153 ± 35
t-(R)α-diMeHA	2,148	19,733 ± 2,913

Blood was taken from groups of four mice killed at various time intervals (1–16 hr) after administration of (R)α-MeHA (24 μmol/kg, p.o.). Blood was withdrawn from six male volunteers at various times (30 min–24 hr) after administration of (R)α-MeHA (1.4 mmol, *i.e.*, ~25 μmol/kg, p.o.) and means ± S.E.M. calculated from each individual A.U.C.

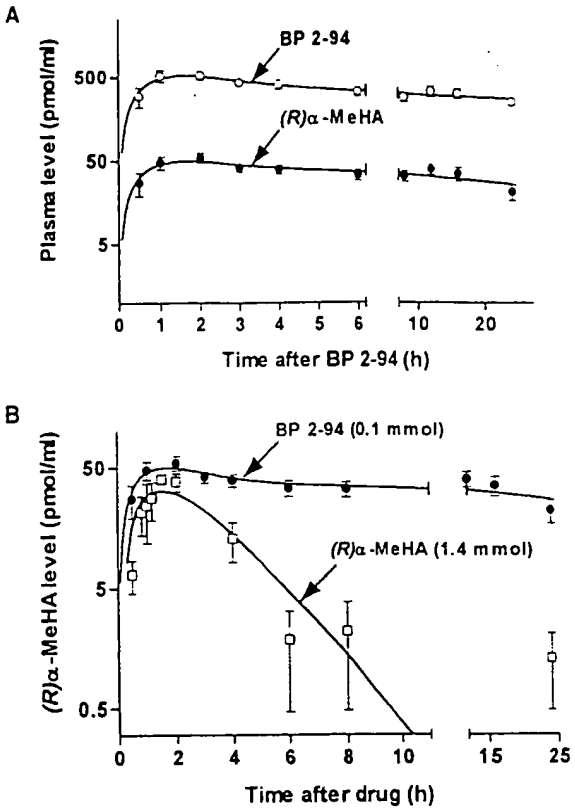


Fig. 4. Compared plasma levels of *(R)*α-MeHA-ir (logarithmic scale) after administration of either *(R)*α-MeHA or BP 2-94 to healthy human volunteers. A, Levels of *(R)*α-MeHA and BP 2-94 after administration of BP 2-94 (0.1 mmol, p.o.) to 12 human volunteers. Means ± S.E.M. B, Levels of *(R)*α-MeHA after administration of either *(R)*α-MeHA (1.4 mmol, p.o.) to 6 human volunteers or BP 2-94 (0.1 mmol, p.o.) to 12 human volunteers. Means ± S.E.M.

the purpose of comparison, and although two distinct populations of volunteers were involved, the changes in plasma *(R)*α-MeHA-ir levels after administration of this compound or its prodrug BP 2-94 are reported together in figure 4B. (Notice that the 6-, 8- and 25-hr time points in the time curve after *(R)*α-MeHA administration are too close to the limit of detection to document a slow terminal elimination). Moreover, the ratio of plasma C_{max} of t-*(R)*α-diMeHA and *(R)*α-MeHA-ir was 0.7 ± 0.2 in human volunteers receiving BP 2-94 (0.05-0.25 μmol), whereas it reached 98 ± 21 in the volunteers who received *(R)*α-MeHA (1.4 mmol).

Effects of BP 2-94 on capsaicin-induced plasma protein extravasation in rat tissues. After capsaicin administration the amount of Evans blue dye (μg/g) raised from 16.3 ± 1.9 to 159.5 ± 12.5, from 13.9 ± 1.8 to 107.9 ± 10.3 and from 6.1 ± 0.5 to 25.5 ± 1.9 μg/g, respectively, within bronchi, esophagus and bladder, representing the percent increases (table 2). Corresponding values were 2.3 ± 0.3 5.3 ± 0.6, 17.5 ± 1.3 and 12.7 ± 1.2 μg/g in skin, conjunctiva nasal mucosa and trachea of controls and increased significantly (3- to 7-fold) after capsaicin (table 2). In dura mater plasma extravasation was evaluated in animals having received FITC-albumin whose levels were 21.2 ± 2.0 μg/g in controls and enhanced by 86% after capsaicin (table 2). Pretreatment with BP 2-94 in increasing doses (0.8-240 μmol/kg) significantly reduced the response to capsaicin in all tissues studied. This inhibitory effect took place dose-dependentl

TABLE 2
Inhibition of capsaicin-induced plasma extravasation by BP 2-94 in various rat tissues

Tissue	Capsaicin Effect (%)	BP 2-94 Inhibitory Effect	
		ED_{50} values (μmol/kg)	Maximal effect (%)
Skin	+787 ± 209	14 ± 8	−48 ± 5
Conjunctiva	+299 ± 61	1.3 ± 0.6	−65 ± 6
Nasal mucosa	+326 ± 49	1.3 ± 0.7	−33 ± 3
Trachea	+614 ± 118	1.3 ± 0.7	−55 ± 5
Bronchi	+882 ± 182	0.8 ± 0.4	−54 ± 4
Esophagus	+678 ± 162	3.0 ± 1.2	−65 ± 5
Urinary bladder	+315 ± 57	0.6 ± 0.1	−63 ± 2
Dura mater	+86 ± 24	3.3 ± 2.0	−87 ± 7

The animals were given increasing doses of BP 2-94 orally 2 hr before capsaicin (90 μg/kg, i.v.) and Evans blue dye (30 mg/kg, i.v.). For estimation of extravasation in dura mater, rats received FITC-albumin 5 min before capsaicin (300 μg/kg, i.v.). Animals were perfused 5 min after capsaicin treatment and tissues were dissected out. The capsaicin effect represents the mean percent increase ± S.E.M. in dye levels in the various tissues. ED_{50} values ± S.E. and maximal effect ± S.E. were calculated from data which are mean of 6 to 37 independent determinations.

with an ED_{50} of 0.8 ± 0.4, 3.0 ± 1.2 and 0.6 ± 0.1 μmol/kg and a maximal reduction of capsaicin-induced extravasation of 54, 65 and 63% in bronchi, esophagus and bladder respectively (fig. 5). The ED_{50} values and maximal effects in the other tissues were derived from similar dose-response curves and are reported in table 2. Administration of thioperamide (51 μmol/kg) 1 hr before BP 2-94 (40 μmol/kg) reversed completely its inhibitory effect in all tissues but thioperamide alone did not significantly affect the capsaicin-induced extravasation. This is shown on figure 6 in bronchi plus trachea (airways). In addition, administration of *(R)*α-MeHA (80 μmol/kg) also inhibited significantly (by 40%) the response to capsaicin in airways, and thioperamide reversed this effect (fig. 6).

Antinociceptive activity of BP 2-94 in mouse. Phenylbenzoquinone-induced writhing was significantly reduced in mice receiving BP 2-94 orally. The antinociceptive activity of the prodrug was dose-related and occurred with an ED_{50} of 0.03 ± 0.01 μmol/kg and a maximal reduction of the writhing score of 70%, a value close to that obtained with aspirin in maximal dosage (556 μmol/kg) (fig. 7). Combination of BP 2-94 and aspirin at moderate dosages elicited an additive reduction of the nociceptive response to PQ (-32 and -39% for BP 2-94 and aspirin, respectively *vs.* -56% when given together). The antinociceptive activity of BP 2-94 (16 μmol/kg) was maximal after 1 hr, still significant after 3 hr and, although not significant, represented a 43% decrease after 6 hr (fig. 8). *(R)*α-MeHA (160 μmol/kg) given orally 1 hr before PQ induced an effect similar to that elicited by a maximal dose of BP 2-94. DMSO itself, the vehicle used for thioperamide and naloxone, reduced the nociceptive effect of PQ by about 50% (writhing score: 11.2 ± 1.9 *vs.* 21.0 ± 2.3 for DMSO and control treated mice, respectively). However, BP 2-94 (0.16 μmol/kg) and aspirin (556 μmol/kg) administered after DMSO were still effective in inhibiting the nociceptive effect of PQ. The antinociceptive effect of BP 2-94 (0.16 μmol/

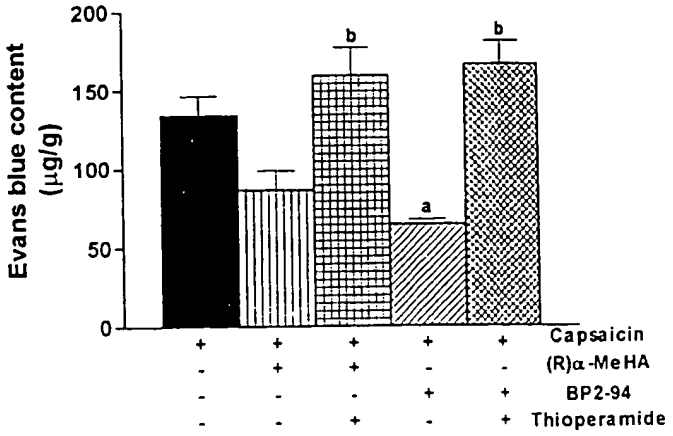


Fig. 6. Thioperamide reversal of the inhibition by *(R)*α-MeHA or BP 2-94 of capsaicin-induced plasma extravasation in rat airways. Thioperamide (51 μmol/kg, i.p.) was administered 1 hr before *(R)*α-MeHA (80 μmol/kg, p.o.) or BP 2-94 (40 μmol/kg, p.o.) and 2.5 hr before capsaicin (90 μg/kg, i.v.) and Evans blue dye (30 mg/kg, i.v.). Animals were perfused 5 min after capsaicin treatment. a, $P < .01$ as compared to capsaicin alone; b, $P < .01$ as compared to capsaicin and *(R)*α-MeHA or BP 2-94. Means ± S.E.M. of 8 to 14 values.

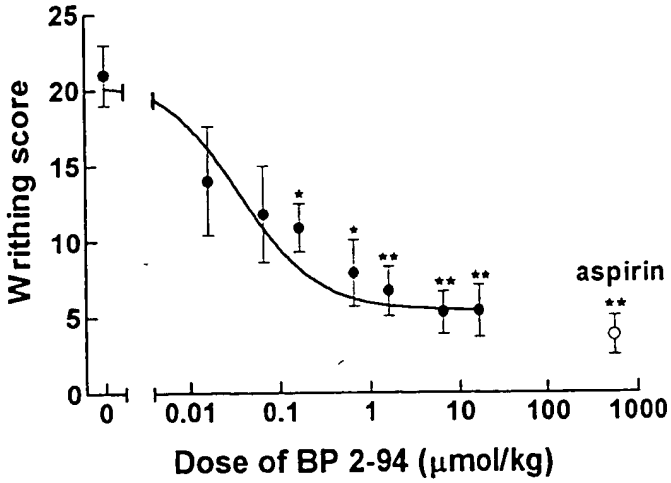


Fig. 7. Antinociceptive activity of BP 2-94 in the phenylbenzoquinone (PQ) writhing test in mouse. PQ (2 mg/kg, i.p.) was administered i.p. 1 hr after BP 2-94 or aspirin (p.o.), and 10 min later the number of writhing episodes were counted for 10 min. *P < .05, **P < .001 as compared to control mice. Means ± S.E.M. of 6 to 45 values.

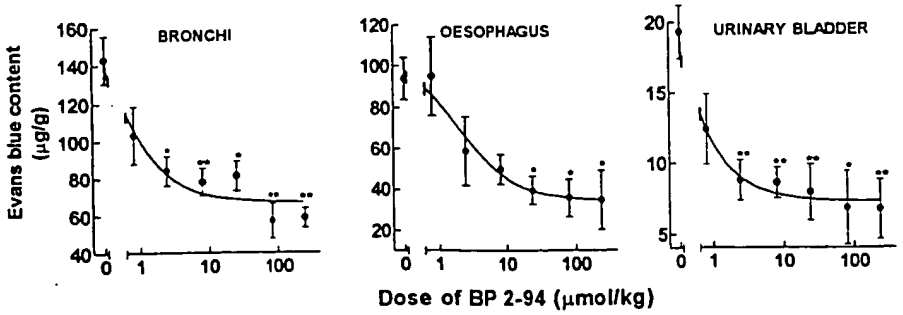


Fig. 5. Effect of BP 2-94 on capsaicin-induced plasma extravasation in rat bronchi, esophagus and urinary bladder. The animals were given BP 2-94 orally at increasing dosages (logarithmic scale) 2 hr before capsaicin (90 μg/kg, i.v.) and Evans blue dye (30 mg/kg, i.v.) and were perfused 5 min after capsaicin. *P < .01, **P < .001. Means ± S.E.M. of 6 to 37 values.

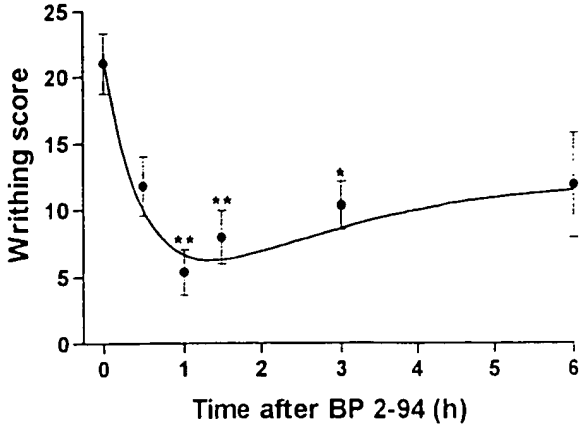


Fig. 8. Kinetics of the antinociceptive effect of BP 2-94 in the phenylbenzoquinone writhing test in mouse. BP 2-94 (16 μmol/kg, p.o.) was given at various times before administration of PQ (2 mg/kg, i.p.). Starting from 10 min after PQ administration, the number of writhing episodes displayed by the animals was counted for 10 min. *P < .05, **P < .01 as compared to control mice. Means ± S.E.M. of 9 to 33 values.

kg) was significantly abolished by previous administration of thioperamide (51 μmol/kg), but not by naloxone (30 μmol/kg). Thioperamide alone did not change the writhing score, and a slight but not significant enhancement of the writhing score could be noticed after naloxone.

In the formalin test, BP 2-94 (16 μmol/kg, p.o.) reduced the duration of licking and biting of the hind paw during both the early (first 5 min) and the late (20-30 min) phase responses after the injection of formalin (fig. 9). BP 2-94 was less efficient than morphine (35 μmol/kg, p.o.) during the early phase but equipotent during the late one.

In the hot plate jump test, BP 2-94 (16 μmol/kg, p.o.) was without effect on the jump latency time whereas morphine (35 μmol/kg, p.o.) increased it significantly by 163% (fig. 10).

Antiinflammatory activity of BP 2-94 in mouse. After preliminary trials the inflammatory effect of zymosan was studied 4 hr after administration of 25 μl of a 0.05% suspension. The inflammatory response to zymosan, *i.e.*, edema, was significantly reduced by about 50% in mouse receiving BP 2-94 (66 μmol/kg, p.o.) at the same time as, before or after zymosan administration. Administered 1 hr before zymosan BP 2-94 decreased in a dose-dependent manner the zymosan-induced edema with an ED_{50} of 1.0 ± 0.4 μmol/kg and a maximal effect of 60%. The antiinflammatory effect of BP 2-94 was significantly abolished by prior administration of thioperamide (51 μmol/kg, i.p.) that alone did not induce significant change (fig. 11).

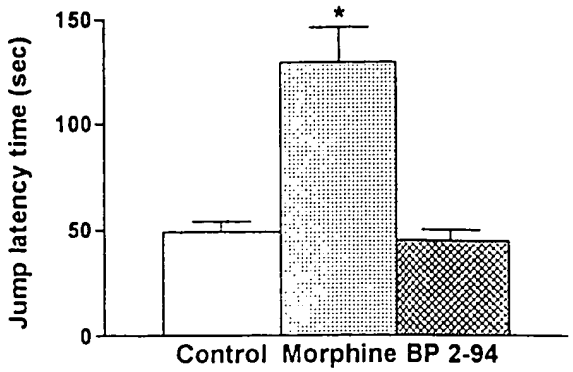


Fig. 10. Effect of BP 2-94 on jump latency in the hot plate test in mouse. Mice were given BP 2-94 (16 μmol/kg, p.o.) or morphine (35 μmol/kg, p.o.) 1 hr before evaluation of the jump latency time. *P < .001 as compared to control or BP 2-94. Means ± S.E.M. of 9 to 10 values.

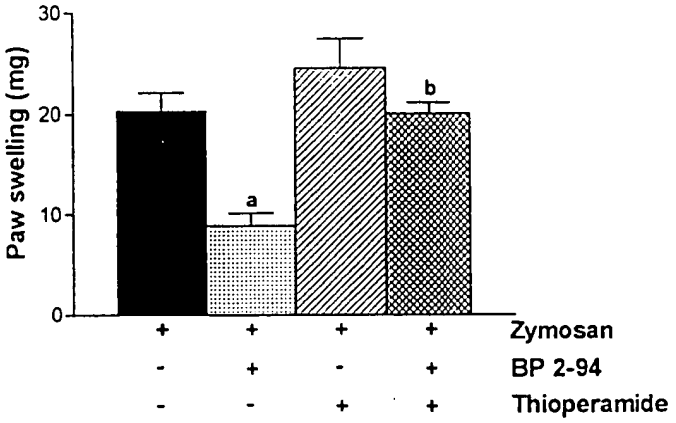


Fig. 11. Thioperamide reversal of the effect of BP 2-94 on zymosan-induced paw edema in mouse. Thioperamide (51 μmol/kg, i.p.) was injected 1 hr before BP 2-94 (1.6 μmol/kg, p.o.) and 2 hr before zymosan injection. a, P < .01 as compared to zymosan, b, P < .001 as compared to BP 2-94. Means ± S.E.M. of 10 values.

Discussion

Our study identifies BP 2-94 as an optimal prodrug of *(R)*αMeHA, enhancing markedly the plasma level of the latter in healthy human volunteers and exerting potent antiinflammatory activity mediated by H_3 receptors on capsaicin-sensitive fibers in rodents.

Initial studies showed that *(R)*α-MeHA displays significantly lower oral bioavailability in humans compared to rodents, as judged from the radioenzymatic assay of plasma levels (table 1). Since much higher plasma levels of t-*(R)*α-diMeHA were detected in humans receiving *(R)*α-MeHA

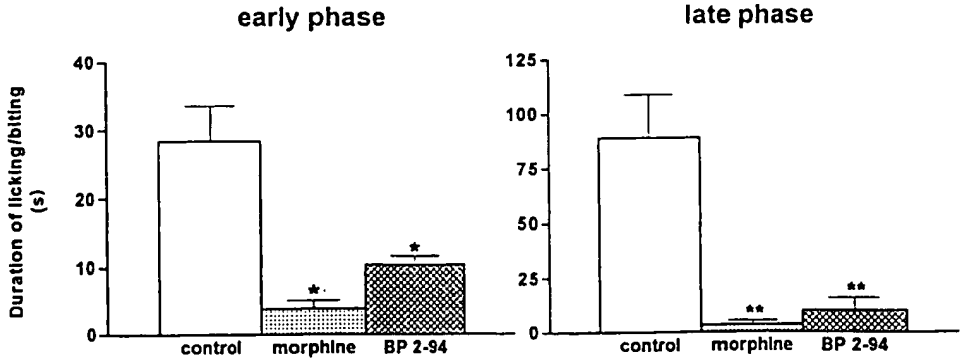


Fig. 9. Antinociceptive activity of BP 2-94 in the mouse formalin test. BP 2-94 (16 μmol/kg, p.o.) or morphine (35 μmol/kg, p.o.) were administered 1 hr before formalin injection into the hind paw. The duration of licking and biting was measured for 5 min after the injection of 20 μl of 0.2% formalin (early phase) or 20 min after 20 μl 1% formalin (late phase). *P < .01, **P < .001. Means ± S.E.M. of 6 to 12 values.

orally, it was hypothesized that this was due to an extensive imidazole ring methylation by the enzyme histamine-N-methyltransferase (EC 2.1.1.8) during the first pass in the liver and leading to an inactive metabolite. In support of this hypothesis *(R)*α-MeHA is readily methylated by the enzyme, and the product is, as with any other ring-substituted compound, lacking any agonist activity at the H_3 receptor (see Arrang *et al.*, 1983; Ganellin *et al.*, 1995; Stark *et al.*, 1994, 1995) (see "Results"). In addition, a much lower hepatic HMT activity is found in the rat (which inactivates HA in peripheral tissues mainly by oxidative deamination) than in many other species including humans (Brown *et al.*, 1959; Hesterberg *et al.*, 1984). To circumvent this difficulty we have explored a strategy based on the design of a series of azomethine derivatives of *(R)*α-MeHA in which the ammonium group, being essential for recognition by HMT (Barth and Lorenz, 1978; Barth *et al.*, 1980) (as well as by the H_3 receptor), is reversibly engaged in a Schiff base, the latter being stabilized by hydrogen bonding with the hydroxyl group of the hydroxybenzophenone moiety (Garbarg *et al.*, 1994; Krause *et al.*, 1995). Such azomethines were previously used in halogenated form as lipophilic prodrugs to promote the brain penetration of GABA (Kaplan *et al.*, 1980; Bergmann, 1985), but apparently were never used before with the aim of protecting a bioactive compound from metabolic degradation.

To identify an optimal prodrug among a large series of azomethine derivatives, *i.e.*, with adequate hydrolysis rate *in vitro*, high oral bioavailability and adequate generation of *(R)*α-MeHA in tissues, we developed a sensitive and specific RIA for this amine after its derivatization according to a principle previously applied to RIAs for HA and t-MeHA (Garbarg *et al.*, 1989a). This assay was also applied to the determination of the level of the prodrug in tissues after its total hydrolysis into *(R)*α-MeHA by heating the tissue extracts in acidic medium.

In agreement with our expectations BP 2-94, a compound selected among a series of related azomethine derivatives of *(R)*α-MeHA, was no longer a substrate for the HA-methylating enzyme. On its unchanged form it did not display any significant H_3 receptor agonist activity, but it was slowly hydrolyzed *in vitro* into the potent agonist *(R)*α-MeHA. This hydrolysis appeared to be essentially of chemical nature because it was not accelerated in the presence of tissue extracts and also occurred at a slow rate *in vivo*, resulting in long-lasting (over 24 hr) plasma levels of the prodrug and *(R)*α-MeHA in human volunteers.

In the latter, 24 hr after an oral dose of 0.1 mmol (about 0.5 mg/kg) of BP 2-94, plasma levels of *(R)*α-MeHA-ir were ~30 nM, *i.e.*, one order of magnitude higher than the EC_{50} of the drug at the H_3 receptor. The success of the prodrug strategy was also shown by the markedly improved human bioavailability of *(R)*α-MeHA when administered orally in form of BP 2-94. The A.U.C. of *(R)*α-MeHA-ir in plasma was approximately 100 times higher in this case.

In addition, in mice receiving BP 2-94 orally, high levels of both the prodrug and *(R)*α-MeHA-ir were found in most tissues, except in the brain where they remained almost undetectable at any time. This was rather unexpected if one takes into account the central effects of GABA prodrugs, which are also azomethine derivatives of 4-hydroxybenzophenone (Kaplan *et al.*, 1980; Jilek *et al.*, 1990) but in halogenated form. In fact, substitution of the hydroxybenzophenone moiety by halogens leads also to prodrugs of *(R)*α-MeHA with markedly enhanced brain penetration as compared with BP 2-94, and which therefore could, in contrast to BP 2-94, be targeted to therapeutic applications resulting from H_3 receptor stimulation in brain (Garbarg *et al.*, 1994; Krause *et al.*, 1995).

In rodents, oral administration of BP 2-94 in low dosage resulted in a series of characteristic and long-lasting responses, all attributable to H_3 receptor activation by slowly released *(R)*α-MeHA. In all tissues tested there was particularly an inhibition of capsaicin-induced plasma protein extravasation, generally by up to 60% (but up to 87% in dura mater), which occurred with widely varying ED_{50} values (table 2). The effect of capsaicin is known to result indirectly from release of proinflammatory neuropeptides, such as tachykinins or CGRP from perivascular peripheral endings of unmyelinated sensory C-fibers (Holzer, 1991; Maggi, 1995). The inhibitory modulatory role of the H_3 receptor on these sensory fibers was first demonstrated on vagal nerve endings in airways (Ichinose and Barnes, 1989a; Ichinose *et al.*, 1989) and then on trigeminal nerve endings in dura mater (Matsubara *et al.*, 1992), in both cases evidenced by plasma protein extravasation. In addition, H_3 receptor mediated inhibition of the immunoreactive substance P release elicited by antidromic stimulation of rat sciatic nerve (Ohkubo *et al.*, 1995) and of CGRP release in heart (Imamura *et al.*, 1996) were demonstrated directly.

These data, together with those of our study, suggest that the H_3 receptor is expressed by C-fibers ending in a large variety of tissues, *i.e.*, not only by sensory cranial nerves but also sensory spinal nerves like those ending in the urinary bladder. The mechanism responsible for the presynaptic inhibition of neuropeptide release could be the same as for the other presynaptic effects of H_3 receptor agonists, *i.e.*, inhibition of calcium ion influx (Arrang *et al.*, 1990) or activation of calcium- or ATP-sensitive potassium channels (Stretton *et al.*, 1992; Ohkubo and Shibata, 1995). These mechanisms are shared by other receptors displaying similar presynaptic localizations and functions, *i.e.*, somatostatin, α_2 adrenergic or opiate receptors (Maggi, 1995). From a physiological point of view the presence of the H_3 receptor on C-fibers has been proposed to reflect its participation in a negative feedback loop controlling the release of mast-cell mediators (including HA) by these closely apposed fibers (Dimitriadou *et al.*, 1994). Via this loop HA release triggered by the secretion of endogenous substance P and/or CGRP is limited by activation of H_3 receptors inhibiting substance P and/or CGRP release (Foreman, 1987; Imamura *et al.*, 1996; Okhubo *et al.*, 1994).

The alleged role of tachykinins retrogradely or anterogradely released from sensory fibers in the mediation of inflammatory and nociceptive responses, respectively, prompted us to test the effect of BP 2-94 in classical rodent models of inflammation and nociception.

Zymosan-induced paw swelling was inhibited via H_3 receptor stimulation after BP 2-94 administration, as shown by the blockade of this effect elicited by thioperamide. The multiple inflammatory responses to zymosan, an insoluble fraction of yeast cell wall, are known to include 1) generation of anaphylatoxins that induce HA release from mast cells, 2) biosynthesis of eicosanoids by neutrophils or macrophages, 3) generation and release of PAF, oxygen free radicals and lysosomal enzymes (Doherty *et al.*, 1985; Rao *et al.*, 1994).

Additional studies are required to establish which of these various inflammatory pathways are modified by H_3 receptor agonists, although it seems likely that the reductions in proinflammatory substance P (Payan, 1989) release and impairment of mast cell reactivity induced by these agents might contribute.

Finally, a marked and long-lasting antinociceptive activity of BP 2-94 in low dosage (ED_{50} = 0.03 ± 0.01 μmol/kg, p.o.), also clearly mediated by the H_3 receptor and independent from endogenous opioids, was evidenced in the PQ-induced writhing test. The maximal effect of BP 2-94, *i.e.*, a 70% reduction in the number of abdominal torsions, was similar to that of aspirin, and the effects of the two agents in moderate dosages were apparently additive.

In the formalin test the antinociceptive effect of BP 2-94 in maximal dosage was less marked than that of morphine during the early phase (which is thought to correspond to direct activation of sensory fibers by formalin) but equivalent to the latter in the late phase, which may correspond to a secondary inflammatory reaction (Kayser and Guilbaud, 1994). In contrast, no significant antinociceptive activity was detected in the hot plate jump test. Taken together these observations suggest that the antinociceptive activity of the H_3 receptor agonist results from an inhibition of nociceptive messages transmission by sensory C-fibers via an action at peripheral sites.

The pattern of peripheral antinociceptive and antiinflammatory actions of the H_3 receptor agonist evidenced here and largely attributable to the widespread inhibition of tachykinin release, suggests novel therapeutic applications for this class of drugs. Tachykinins modulate the activity of a number of different leukocytes involved in both acute and delayed inflammatory responses and may play a role in the pathogenesis of such diverse diseases as arthritis, asthma and inflammatory bowel diseases (Payan, 1989). The applications of H_3 receptor agonists might be more general than those of tachykinin receptor antagonists that are generally specific for a single receptor subtype. In addition, the application of these drugs in asthma is further supported by their inhibitory effect on vagally induced bronchoconstriction (Barnes, 1992). In migraine and related disorders, their efficacy might derive from the inhibition of release of both proinflammatory tachykinins and vasodilatory CGRP from trigeminal nerve endings, a mechanism proposed to account for the therapeutic efficacy of sumatriptan (Buzzi *et al.*, 1991; Moskowitz, 1991). These various hypotheses are currently tested in ongoing clinical trials with BP 2-94 due to the good plasma level of this compound. Finally, it should be stressed that among antiinflammatory agents H_3 receptor agonists display the interesting and unique property of decreasing gastric acid secretion (Bertaccini and Coruzzi, 1995) and exerting antiulcer activity (Morini *et al.*, 1995) in relation with the inhibition of HA release from enterochromaffin-like cells (Prinz *et al.*, 1993; Soldani *et al.*, 1996).

Acknowledgments

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International Union of Pharmacology. XIII. Classification of Histamine Receptors

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I. Introduction and Historical Perspective

The classification of histamine receptors has to date been based on rigorous classical pharmacological analysis, and as yet, the classification of the three histamine receptors that have been defined by this process, (i.e., the H_1-, H_2-, and H_3-receptors) have not been added to because of more recent molecular biological approaches (Schwartz et al., 1991, 1995; Hill, 1990; Leurs et al., 1995b). The scant number of known histamine receptors, compared with the plethora of receptors for some other endogenous substances, probably reflects the relative neglect of histamine rather than a paucity of its receptors. There is some preliminary evidence of heterogeneity of the known histamine receptors (which will be reviewed later in this article), but the acceptance of additional subtypes still awaits the identification of "sequence differences" within a single species and the development of selective agonists and antagonists providing the structural, recognition, and transductional information necessary for reliable classification.

The first histamine receptor antagonists (popularly referred to as the classical antihistamines but now called H_1-receptor antagonists) were synthesized (Bovet and Staub, 1936; Bovet, 1950) over 20 years after the discovery (Barger and Dale, 1910) and descriptions of some of the physiological effects (Dale and Laidlaw, 1910) of histamine. These accomplishments had been preceded, as for some other endogenous biogenic amines, by its synthesis as a chemical curiosity (Windaus and Vogt, 1907). Early studies of the antihistamines were qualitative, for example, the demonstration of their ef-

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fectiveness in protecting against bronchospasm produced in guinea pigs by anaphylaxis or administration of histamine (Bovet and Staub, 1936). Though qualitative, these studies yielded compounds, e.g., mepyramine (pyrilamine), that remain major ligands to define histamine receptors.

These antagonists were shown to reduce the effects of histamine on many tissues, notably vascular and extravascular smooth muscle (e.g., guinea pig ileum), but it became apparent that some of the effects of histamine were refractory to these classical antihistamines (Loew, 1947). For example, histamine-stimulated gastric secretion was shown to be unresponsive to three different antihistamines (Ashford et al., 1949). The vasodilator response to histamine in the cat was shown to be only partly sensitive to an antihistamine, leading to the suggestion that histamine causes vasodilatation by combining with more than one receptor (Folkow et al., 1948). The application of the method of Schild (Arunlakshana and Schild, 1959) to the classification of receptors revealed that the pA_2 ($-\log K_B$) value of mepyramine for antagonism of the positive chronotropic effect of histamine on the right atrium of the guinea pig differed from mepyramine's pA_2 value for antagonism of the contractile response to histamine in guinea pig ileum, implying that the receptors involved were distinct (Arunlakshana and Schild, 1959; Trendelenburg, 1960). The histamine receptor in guinea pig ileum and in other tissues that showed the same or similar pA_2 value for these early antihistamines was then named the H_1-receptor (Ash and Schild, 1966). As the relative potencies of these histamine antagonists and histamine agonists on gastric acid secretion, relaxation of rat uterus, and chronotropy of the guinea pig right atrium differed from those on the H_1-receptor, it was concluded that a separate histamine receptor was involved in these responses.

The development of specific antagonists (H_2-antagonists) for this novel receptor represents a classic example of rational drug design (Black et al., 1972; Black, 1989) and showed the "practical value" (Green and Maayani, 1987; Jenkinson, 1987) of a quantitative approach to the analysis of receptor antagonism (Arunlakshana and Schild, 1959). Burimamide was the first compound to be described (Black et al., 1972) that had a higher pA_2 for antagonism of the histamine-mediated responses on guinea pig atrium and rat uterus than the pA_2 determined for antagonism of the contractile response to histamine in guinea pig ileum. Burimamide was also able to reduce gastric acid secretion in dogs and humans and to reduce the blood pressure response of the cat to histamine (Black et al., 1972). A large number of more potent and selective H_2-receptor antagonists have since been developed (Cooper et al., 1990), although further quantitative investigations of the antagonist potency of burimamide on other histamine-mediated responses contributed to the definition and classification of the histamine H_3-receptor (Arrang et al., 1983).

The third histamine receptor was also defined by a functional assay. Histamine was found to inhibit its own synthesis and release in rat cerebral cortical slices, and the effects of H_1- and H_2- receptor agonists and antagonists indicated a distinct receptor (Arrang et al., 1983, 1987b). A highly selective agonist, R-(α)-methylhistamine, and antagonist, thioperamide, clearly defined the H_3-receptor (Arrang et al., 1987). Since that time, considerable efforts have been made to develop other H_3-receptor–selective agonists and antagonists (Garbarg et al., 1992; Jansen et al., 1992; Van der Goot et al., 1992; Vollinga et al., 1994; Ganellin et al., 1995; Ligneau et al., 1995; Stark et al., 1996b,c).

Table 1 summarizes some of the operational characteristics used to define the nature of the histamine receptor involved in different tissue responses. Histamine derivatives are numbered according to the system given in figure 1 (Black and Ganellin, 1974).

II. Histamine H_1-Receptor

A. Distribution and Function

The study of the distribution of histamine H_1-receptors in different mammalian tissues has been greatly aided by the development of selective radioligands for this particular histamine receptor subtype. [^{3}H]mepyramine was originally developed in 1977 (Hill et al., 1977) and since that time has been used successfully to detect H_1-receptors in a wide variety of tissues including: mammalian brain; smooth muscle from airways, gastrointestinal tract, genitourinary system, and the cardiovascular system; adrenal medulla; and endothelial cells and lymphocytes (Hill, 1990). In some tissues and cells, however, it is notable that [^{3}H]mepyramine additionally binds to secondary non-H_1–receptor sites (Chang et al., 1979a; Hill and Young, 1980; Hadfield et al., 1983; Mitsuhashi and Payan, 1988; Arias-Montano and Young, 1993; Dickenson and Hill, 1994; Leurs et al., 1995b). In rat liver, in which [^{3}H]mepyramine predominantly binds to a protein homologous with debrisoquine 4-hydroxylase cytochrome P450 (Fukui et al., 1990), quinine can be used to inhibit this nonspecific binding. This observation has led Liu et al. (1992) to suggest that quinine may be used to inhibit binding to other lower affinity sites. However, it is clear that not all secondary binding sites for [^{3}H]mepyramine are sensitive to inhibition by quinine (Dickenson and Hill, 1994). Thus, in DDT_1MF-2 cells, a 38 to 40 kDa protein has been isolated, which binds H_1-receptor antagonists with K_D values in the micromolar range (Mitsuhashi and Payan, 1988, Mitsuhashi et al., 1989) but which is not sensitive to inhibition by quinine (Dickenson and Hill, 1994). Nevertheless, DDT_1MF-2 cells can be shown to additionally possess [^{3}H]mepyramine binding sites that have the characteristics of H_1-receptors (i.e., K_D values in the nanomolar range) and to mediate functional responses, which are clearly produced by histamine H_1-receptor

TABLE 1
Operational characteristics of histamine receptors

Receptor	Location	Response	Agonists	Antagonists
Histamine H_1	Most smooth muscle, endothelial cells, adrenal medulla, heart, CNS	Smooth muscle contraction, stimulation of NO formation, endothelial cell contraction, increased vascular permeability, stimulation of hormone release, negative inotropism, depolarization (block of leak potassium current) and increased neuronal firing, inositol phospholipid hydrolysis and calcium mobilization, hyperpolarization by Ca^{2+}-dependent potassium current	Histamine[a] 2-[3-(Trifluoromethyl)-phenyl]histamine 2-Thiazolylethylamine 2-Pyridylethylamine 2-Methylhistamine	Mepyramine (+) and (−) Chlorpheniramine Triprolidine Temelastine Diphenhydramine Promethazine
Histamine H_2	Gastric parietal cells, vascular smooth muscle, suppressor T cells, neutrophils, CNS, heart, uterus (rat)	Stimulation of gastric acid secretion, smooth muscle relaxation, stimulation of adenylyl cyclase, positive chronotropic and inotropic effects on cardiac muscle, decreased firing rate, hyperpolarization or facilitation of signal transduction in CNS, block of Ca^{2+}-dependent potassium conductance (I AHP, accommodation of firing, after-hyperpolarization), increase of hyperpolarization-activated current, inhibition of lymphocyte function	Histamine[a] Amthamine Dimaprit Impromidine[b] Arpromidine[b]	Cimetidine Ranitidine Tiotidine Zolantidine Famotidine
Histamine H_3	CNS, peripheral nerves (heart, lung, gastrointestinal tract), endothelium, enterochromaffin cells	Inhibition of neurotransmitter release, endothelium-dependent relaxation of rabbit middle cerebral artery, inhibition of gastric acid secretion (dog), increase in smooth muscle voltage-dependent Ca^{2+} current, inhibition of firing of tuberomammilary (histaminergic) neurons	Histamine[a] R-α-methylhistamine Imetit Immepip N^{α}-methylhistamine[a]	Thioperamide Clobenpropit Iodophenpropit Iodoproxyfan

CNS, central nervous system.
[a] Nonselective.
[b] H_3-antagonist.

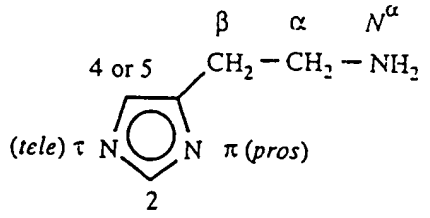


FIG. 1. Numbering for histamine derivatives.

activation (Dickenson and Hill, 1992; White et al., 1993; Dickenson and Hill, 1994).

Other radioligands that have been used to study histamine H_1-receptors are [^{3}H]mianserin (Peroutka and Snyder, 1981), [^{3}H]doxepin (Tran et al., 1981; Kamba and Richelson, 1984; Taylor and Richelson, 1982), [^{125}I]iodobolpyramine (Bouthenet et al., 1988), [^{125}I]iodoazidophenpyramine (Ruat et al., 1988), and [^{3}H](+)-N-methyl-4-methyldiphenhydramine (Treherne and Young, 1988b). [^{125}I]Iodobolpyramine has been used for autoradiographic localization of H_1-receptors in guinea pig brain, although less success has been achieved in rat brain (Körner et al., 1986; Bouthenet et al., 1988). The very slow dissociation of [^{3}H]mepyramine from H_1-receptors at low temperatures (e.g., 4°C) does, however, mean that this ligand can also be used for autoradiography (Palacios et al., 1981a,b; Rotter and Frostholm, 1986). [^{125}I]Iodoazidophenpyramine is a very potent H_1-receptor antagonist that can bind irreversibly to H_1-receptors following irradiation with ultraviolet light (Ruat et al., 1988). [^{11}C]Mepyramine and [^{11}C]doxepin have also proved useful for imaging histamine H_1-receptors in the living human brain (Villemagne et al., 1991; Yanai et al., 1992, 1995).

H_1-receptors have been extensively studied in blood vessels (Barger and Dale, 1910; Dale and Laidlaw, 1910; Folkow et al., 1948; Black et al., 1972) and other smooth muscle preparations (Ash and Schild, 1966; Black et al., 1972; Marshall, 1955; Hill, 1990). In smooth muscles, such as the guinea pig ileum, which freely generate muscle action potentials, modulation of action-potential discharge by low concentrations of histamine is an important mechanism by which tension is increased (Bolton, 1979; Bolton et al., 1981; Bülbring and Burnstock, 1960). In guinea pig ileum, there is also evidence

that a component of the contractile response to histamine is mediated by inositol 1,4,5-trisphosphate–induced mobilization of intracellular calcium (Morel et al., 1987; Bolton and Lim, 1989; Donaldson and Hill, 1986b). In nonexcitable smooth muscles, such as airway and vascular smooth muscle, contractile responses to H_1-receptor stimulation primarily involve mobilization of calcium from intracellular stores as a consequence of inositol phospholipid hydrolysis (Matsumoto et al., 1986; Kotlikoff et al., 1987; Takuwa et al., 1987; Hall and Hill, 1988; Paniettieri et al., 1989; Van Amsterdam et al., 1989).

In vascular endothelial cells, H_1-receptor stimulation leads to several cellular responses including: (*a*) changes in vascular permeability (particularly in postcapillary venules) as a result of endothelial cell contraction (Majno and Palade, 1961; Majno et al., 1968; Meyrick and Brigham, 1983; Grega, 1986; Killackey et al., 1986; Svensjo and Grega, 1986); (*b*) prostacyclin synthesis (McIntyre et al., 1985; Brotherton, 1986; Carter et al., 1988; Resink et al., 1987); (*c*) synthesis of platelet-activating factor (McIntyre et al., 1985); (*d*) release of Von Willebrand factor (Hamilton and Sims, 1987); and (*e*) release of nitric oxide (Van De Voorde and Leusen, 1993; Toda, 1984). The H_1-receptor has also been characterized on human T lymphocytes using [^{125}I]iodobolpyramine (Villemain et al., 1990) and shown to increase $[Ca^{2+}]_i$ (Kitamura et al., 1996).

Histamine H_1-receptors have long been established to be present in the adrenal medulla and to elicit the release of catecholamines (Emmelin and Muren, 1949; Staszewska-Barczak and Vane, 1965; Robinson, 1982; Livett and Marley, 1986; Noble et al., 1988). Thus, histamine can induce the release of both adrenaline and noradrenaline from cultured bovine adrenal chromaffin cells (Livett and Marley, 1986). In these cells, histamine can also stimulate phosphorylation of the catecholamine biosynthesis enzyme tyrosine hydroxylase via a mechanism that involves release of intracellular calcium (Bunn et al., 1995). In addition to its effects on catecholamine synthesis and release from adrenal chromaffin cells, histamine can also elicit the release of leucine- and methionine-enkephalin (Bommer et al., 1987). Furthermore, after prolonged exposure to histamine, there is a marked increase in messenger ribonucleic acid-encoding proenkephalin A (Bommer et al., 1987; Kley, 1988; Wan et al., 1989).

In human atrial myocardium and guinea pig ventricle, histamine produces negative inotropic effects (Guo et al., 1984; Genovese et al., 1988; Zavecz and Levi, 1978). In human myocardium, this response is associated with inhibitory effects on heart rate and can be unmasked when the positive effects of histamine on the rate and force of contraction (mediated via H_2-receptors) are attenuated by conjoint administration of adenosine or adenosine A_1-receptor agonists (Genovese et al., 1988). However, in guinea pig left atria (Reinhardt et al., 1974, 1977; Steinberg and Holland, 1975; Hattori et al., 1983, 1988a) and rabbit papillary muscle (Hattori et al., 1988b), histamine produces a positive inotropic response via a mechanism that is not associated with a rise in adenosine 3c,5c-cyclic monophosphate (cAMP[b]) levels (see Hill, 1990).

Histamine H_1-receptors are widely distributed in mammalian brain (Hill, 1990; Schwartz et al., 1991). In human brain, higher densities of H_1-receptors are found in neocortex, hippocampus, nucleus accumbens, thalamus, and posterior hypothalamus, whereas cerebellum and basal ganglia show lower densities (Chang et al., 1979b; Kamba and Richelson, 1984; Martinez-Mir et al., 1990; Villemagne et al., 1991; Yanai et al., 1992). The distributions in rat (Palacios et al., 1981a) and guinea pig (Palacios et al., 1981b; Bouthenet et al., 1988) are similar to each other and to humans with the exception that the guinea pig cerebellum shows high density (Ruat and Schwartz, 1989; Chang et al., 1979b; Hill and Young, 1980; Palacios et al., 1981b; Bouthenet et al., 1988). In most brain areas, there was overlap of H_1-receptor binding sites and messenger ribonucleic acid levels except in hippocampus and cerebellum in which the discrepancy is likely to reflect the presence of abundant H_1-receptors in dendrites of pyramidal and Purkinje cells, respectively (Traiffort et al., 1994). Histamine H_1-receptor activation causes inhibition of firing and hyperpolarization in hippocampal neurons (Haas, 1981) and an apamine-sensitive outward current in olfactory bulb interneurons (Jahn et al., 1995), effects most likely produced by intracellular Ca^{2+} release. However, many other notably vegetative ganglia (Christian et al., 1989), hypothalamic supraoptic (Haas et al., 1975), brainstem (Gerber et al., 1990; Khateb et al., 1990), thalamic (McCormick and Williamson, 1991), and human cortical neurons (Reiner and Kamondi, 1994) are excited by histamine H_1-receptor activation through a block of a potassium conductance.

B. H_1-Selective Ligands

Although a large number of compounds have been synthesized as selective and competitive antagonists of the histamine H_1-receptor (see for example Casy, 1977; Ganellin, 1982), chemical effort directed at the generation of highly potent and selective H_1-receptor agonists has not achieved the same success. Modification of the ethylamine side chain of histamine is not favorable for H_1-receptor agonism (Leurs et al., 1995b). Furthermore, resolution of the enantiomers of the chiral compounds generated by methylation of the α- or β-positions did not reveal any stereoselectivity of the side chain for the H_1-receptor (Arrang et al., 1987; Leurs et al., 1995). Alkylation of the side chain amine group does not dras-

[b] Abbreviations: cAMP, cyclic adenosine 3c,5c-cyclic monophosphate; cNDA, complementary deoxyribonucleic acid; CNS, central nervous system; DPPE, N-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine; GTPγS, guanosine 5′*O*-(3-thiotriphosphate); NMDA, N-methyl-D-aspartate; TM, transmembrane.

tically reduce H_1-receptor activity, but N^{α}- and N^{α},N^{α}-dimethylhistamine are also potent agonists for the H_3-receptor (table 2; fig. 2; Arrang et al., 1983). Modification of the imidazole moiety of histamine has been the most successful approach for obtaining agonists with selectivity for the H_1-receptor. Replacement of the imidazole moiety of histamine by other aromatic heterocyclic ring structures in 2-pyridylethylamine and 2-thiazolylethylamine yields two compounds with selectivity for the H_1-receptor (table 2; fig. 2). Both compounds act as full agonists in producing contraction of guinea pig ileum (Donaldson and Hill, 1986c), but in other tissues (e.g., guinea pig cerebral cortical slices or DDT_1MF-2 cells), 2-pyridylethylamine behaves as a low-efficacy agonist (Donaldson and Hill, 1986a; White et al., 1993). Substitutions in the 2-position of the imidazole ring of histamine have produced compounds that are the most selective H_1-agonists available (Zingel et al., 1995). Thus, 2(3-bromophenyl)histamine and 2[3-(trifluoromethyl)-phenyl]histamine are both relatively potent and highly selective H_1-agonists (table 2; fig. 2; Leschke et al., 1995). Both compounds appear to be potent H_1-agonists in guinea pig ileum (Leschke et al., 1995), although some of the halogenated 2-phenylhistamines are low-efficacy agonists in DDT_1MF-2 cells (Zingel et al., 1990; White et al., 1993) and in guinea pig aorta (Leschke et al., 1995) and can exhibit partial agonist properties.

TABLE 2
Agonist potency ratios of histamine receptors

Histamine	H_1 100	H_2 100	H_3 100
2-(3-(Trifluoromethyl)phenyl)-histamine	128[a]	<0.1[a]	n.d.
2-(3-Bromophenyl)histamine	112[a]	<0.1[a]	n.d.
N^{α}-methylhistamine	72[b]	74[c]	270[d]
2-(2-Thiazolyl)ethylamine	26[b]	2.2[b]	<0.008[d]
2-Methylhistamine	17[c]	4[c]	<0.08[d]
2-(2-Pyridyl)ethylamine	5.6[b]	2.5[b]	n.d.
Arpromidine	Antagonist[e]	10,230[e]	
Impromidine	Antagonist[f]	4,810[f]	Antagonist[d,o]
Sopromidine	2[g]	740[g]	Antagonist[h,p]
Amthamine	1[i]	150[i]	0.002[i]
Dimaprit	<0.0001[j]	71[j]	Antagonist[d,q]
4-Methylhistamine	0.2[c]	43[c]	<0.008[d]
Imetit	<0.1[k]	0.6[k]	6200[k]
Immepip	n.d.	n.d.	2457[l]
R-α-methylhistamine	0.5[m]	1[m]	1550[m]
S-α-methylhistamine	0.5[b]	1.7[b]	13[m]
R-α,S-β-dimethylhistamine	0.07[n]	0.1[n]	1800[n]

Values determined from guinea pig ileum contraction (H_1), guinea pig atrium (chronotropic stimulation, H_2), and inhibition of K^+-stimulated histamine release from rat cerebral cortical slices (H_3), or inhibition of electrically stimulated contraction of guinea pig ileum (H_3). n.d., not determined.

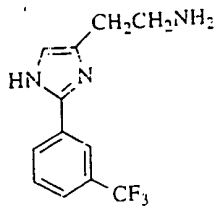
[a] Leschke et al. (1995) [g] Elz et al. (1989) [m] Arrang et al. (1987)
[b] Ganelin (1982) [h] Arrang et al. (1985c) [n] Lipp et al. (1992)
[c] Black et al. (1972) [i] Eriks et al. (1992) [o] See table 4.
[d] Arang et al. (1983) [j] Durant et al. (1977) [p] K_B = 56 nM.
[e] Sellier et al. (1992) [k] Garbarg et al. (1992) [q] K_B = 3 μM.
[f] Durant et al. (1978) [l] Vollinga et al. (1994)

Mepyramine (also known as pyrilamine) is the reference selective and high-affinity H_1-receptor antagonist (table 3; Hill, 1990). Other classical H_1-antagonists that have been used for characterization purposes include chlorpheniramine, tripelennamine, promethazine, and diphenhydramine (fig. 3). Some of these, however, possess marked muscarinic receptor antagonist properties (Hill, 1990, 1987), and consequently the selectivity of these compounds between the three different histamine receptors (table 3) does not guarantee an unambiguous characterization. This can only be achieved by appropriate quantitative assessment of receptor antagonism, preferably with a range of compounds of very different chemical structure. The stereoisomers of chlorpheniramine are particularly useful in this regard (table 3). The enantiomers of 4-methyl-diphenhydramine and brompheniramine also differ by two orders of magnitude in their affinity for the H_1-receptor (Chang et al., 1979b; Treherne and Young, 1988b). The geometric isomer trans-triprolidine is three orders of magnitude more potent than its cis counterpart and is one of the most potent H_1-antagonists available for the guinea pig H_1-receptor (tables 3 and 4; Ison et al., 1973). The tricyclic antidepressants amitriptyline and doxepin are also very potent H_1-receptor antagonists (K_D 0.6 and 0.1 nM respectively; Figge et al., 1979; Aceves et al., 1985).

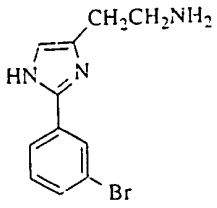
At therapeutic dosages, many of the classical H_1-antihistamines give rise to sedative side effects that have been attributed to occupancy of H_1-receptors in the central nervous system (CNS) (Schwartz et al., 1981; Nicholson et al., 1991; Leurs et al., 1995b). Most of the classical H_1-antihistamines, including promethazine and (+)-chlorpheniramine, readily cross the blood-brain barrier. However, several compounds that penetrate poorly into the CNS and appear to be devoid of central depressant effects are now available (fig. 4). These include terfenadine (Rose et al., 1982; Wiech and Martin, 1982), astemizole (Laduron et al., 1982; Niemegeers et al., 1982), mequitazine (Uzan and Le Fer, 1979), loratadine (Ahn and Barnett, 1986), acrivastine (Leighton et al., 1983; Cohen et al., 1985), cetirizine (Timmerman, 1992b), and temelastine (Brown et al., 1986; Calcutt et al., 1987). The pK_i values for these agents are given in table 5 (Ter Laak et al., 1994).

C. Receptor Structure

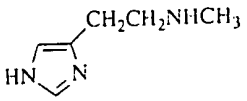
Photoaffinity binding studies using [^{125}I]iodoazidophenpyramine and subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis have indicated that the H_1-receptor protein has a molecular weight of 56 kDa under reducing conditions in rat, guinea pig, and mouse brain (Ruat et al., 1988, 1990b; Ruat and Schwartz, 1989). Similarly, studies in bovine adrenal medullar membranes with another photoaffinity ligand [^{3}H]azidobenzamide (Yamashita et al., 1991b)



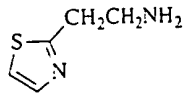
2-(3-Trifluoromethyl phenyl)histamine



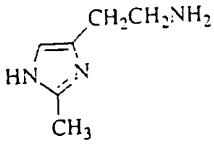
2-(3-Bromophenyl)-histamine



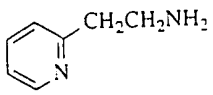
N^{α}-Methylhistamine



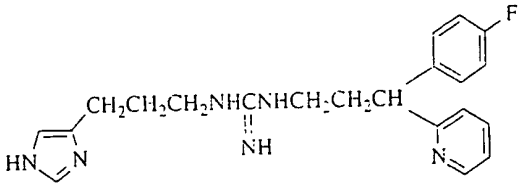
2-(2-Thiazolylethyl)amine



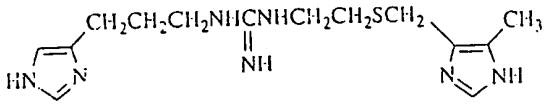
2-Methylhistamine



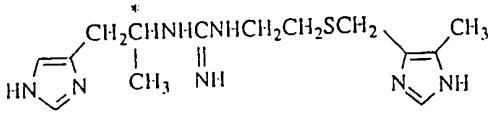
2-(2-Pyridylethyl)amine



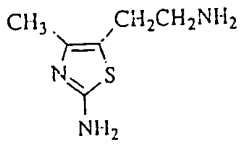
Arpromidine



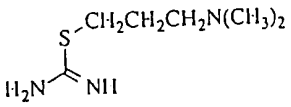
Impromidine



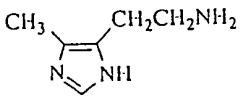
Sopromidine (* :R chirality)



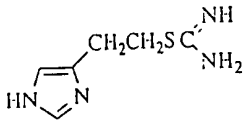
Amthamine



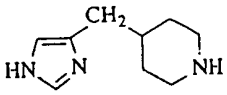
Dimaprit



4-Methylhistamine



Imetit



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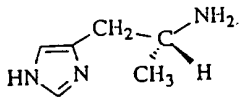
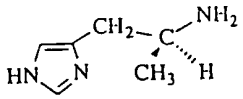
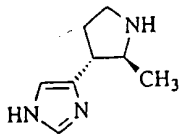


(R)-α-Methylhistamine



(S)-α-Methylhistamine



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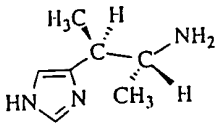


(Rα, Sβ)-α,β-Dimethylhistamine

FIG. 2. Histamine receptor agonists (H_1, H_2, and H_3).

found labeled peptides in the size range 53 to 58 kDa. Interestingly, the specifically labeled H_1-receptor (with [^{125}I]iodoazidophenpyramine) in guinea pig heart was found to have a substantially higher molecular weight, although there is no obvious difference in the pharmacological characteristics of the H_1-receptor in this tissue (Ruat et al., 1990a).

The bovine adrenal medulla H_1-receptor was cloned in 1991 by expression cloning in the *Xenopus* oocyte system (Yamashita et al., 1991a). The deduced amino acid se-

TABLE 3
Antagonist dissociation constants at histamine receptors

	K_B values		
	H_1	H_3	H_2
Doxepin	0.06 nM[a]	n.d.	n.d.
Triprolidine (trans)	0.1 nM[b]	n.d.	n.d.
Temelastine	0.3 nM[c]	>10 μM[c]	n.d.
Mepyramine (pyrilamine)	0.4 nM[d]	5.2 μM[e]	>1 μM[f]
(+)-Chlorpheniramine	0.4 nM[g]	1.2 μM[h]	>58 nM[i]
(−)-Chlorpheniramine	204 nM[g]	1.2 μM[h]	>58 nM[i]
Diphenhydramine	1.0 nM[j]	n.d.	n.d.
Promethazine	1.2 nM[k]	3.0 μM[l]	n.d.
Chlorpromazine	1.2 nM[k]	5.9 μM[l]	n.d.
Tripelenamine	3.2 nM[d]	n.d.	n.d.
Arpromidine	20 nM[m]	agonist[ah]	
Cimetidine	450 μM[n]	800 nM[n]	33 μM[i]
Metiamide	n.d.	920 nM[o]	2.5 μM[p]
Ranitidine	>100 μM[p]	200 nM[p]	>1.2 μM[i]
Famotidine	n.d.	17 nM[q]	n.d.
Zolantidine	6.2 μM[r]	25 nM[r]	>10 μM[s]
Mifentidine	>24 μM[t]	24 nM[t]	100 nM[s]
Tiotidine	>30 μM[u]	15 nM[v]	>12 μM[i]
Iodoaminopotentidine	1.1 μM[w]	2.5 nM[w]	n.d.
Impromidine	3.4 μM[x]	agonist[x]	65 nM[i]
Burimamide	320 μM[y]	7.8 μM[z]	70 nM[i]
Thioperamide	>100 μM[aa]	>10 μM[aa]	4 nM[aa]
Iodophenpropit	n.d.	n.d.	0.25 nM[ab]
Clobenpropit	>10 μM[ac]	>10 μM[ac]	0.13 nM[ac]
Iodoproxyfan	1.4 μM[ad]	5.3 μM[ad]	5 nM[ad, ag]
Impentamine	126 μM[ae]	250 μM[ae]	4 nM[ae]
GR174737	>10 μM[af]	>10 μM[af]	8 nM[af]

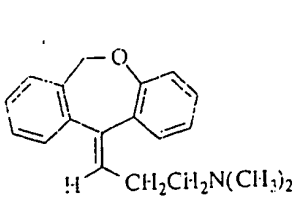
Values determined in functional assays from guinea pig ileum contraction (H_1), biochemical determinations in guinea pig cerebral cortical slices (H_1), chronotropic responses in guinea pig atria (H_2), cyclic AMP accumulation in guinea pig hippocampal slices (H_2), inhibition of histamine release in rat cerebral cortical slices (H_3), and inhibition of transmurally stimulated guinea pig ileum (H_3). n.d., not determined.

[a] Figge et al. (1979)
[b] Ison et al. (1973)
[c] Brown et al. (1986)
[d] Marshall (1955)
[e] Trendelenburg (1960)
[f] Hew et al. (1990)
[g] Hill et al. (1981)
[h] Hill (1990)
[i] Arrang et al. (1983)
[j] Ganellin (1982)
[k] Hill and Young (1981)
[l] Tuong et al. (1980)
[m] Sellier et al. (1992)
[n] Brimblecombe et al. (1975)
[o] Black et al. (1974)
[p] Cavanagh et al. (1983)
[q] Takeda et al. (1982)
[r] Calcutt et al. (1988)
[s] Schwartz et al. (1990)
[t] Donetti et al. (1984)
[u] Donaldson et al. (1988)
[v] Yellin et al. (1979)
[w] Hirschfeld et al. (1992)
[x] Durant et al. (1978)
[y] Buschauer et al. (1992)
[z] Black et al. (1972)
[aa] Arrang et al. (1987)
[ab] Jansen et al. (1992)
[ac] Van der Goot et al. (1992)
[ad] Ligneau et al. (1994)
[ae] Vollinga et al. (1995)
[af] Clitherow et al. (1996)
[ag] Stark et al. (1996a)
[ah] Buschauer (1989)

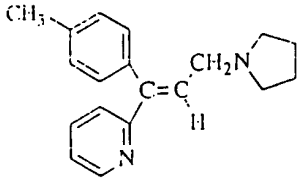
quence represents a 491 amino acid protein with a calculated molecular weight of 56 kDa (table 6). The protein has the seven putative transmembrane (TM) domains expected of a G-protein–coupled receptor and possesses N-terminal glycosylation sites. A striking feature of the proposed structure is the very large third intracellular loop (212 amino acids) and relatively short (17 amino acids) intracellular C terminal tail. The availability of the bovine sequence and lack of introns has enabled the H_1-receptor to be cloned from several species (table 6) including rat (Fujimoto et al., 1993), guinea pig (Horio et al., 1993; Traiffort et al., 1994), mouse (Inove et al., 1996), and human (De Backer et al., 1993; Fukui et al., 1994; Moguilevsky et al., 1994; Smit et al., 1996c). The human histamine H_1-receptor gene has now been localized to chromosome 3 bands 3p14-p21 (Le Coniat et al., 1994).

At the present time, these different clones should be regarded as true species homologues of the histamine H_1-receptor, even though there are notable differences between them in some antagonist potencies (table 4). Unfortunately, the number of H_1-receptor antagonists evaluated in binding studies in cells transfected with the different recombinant receptors is rather limited. Nevertheless, it is clear that the stereoisomers of chlorpheniramine show marked differences between species. For example, the guinea pig H_1-receptor has a K_D of 0.9 nM for (+)-chlorpheniramine, whereas for the rat H_1-receptor, the value is nearer 8 nM (table 4). Similar differences for this compound and others (notably mepyramine and triprolidine) have been reported for the native H_1-receptors in guinea pig and rat brain, respectively (table 4; Chang et al., 1979b; Hill and Young, 1980; Hill, 1990). Such species differences may also explain why [^{125}I]iodobolpyramine can label guinea pig CNS H_1-receptors but is unable to detect H_1-receptors in rat brain (Körner et al., 1986; Bouthenet et al., 1988). The native H_1-receptor protein has been solubilized from both guinea pig and rat brain membranes (Toll and Snyder, 1982; Treherne and Young, 1988a), and the solubilized receptor retains the same differences in H_1-antagonist potency for (+)-chlorpheniramine as that observed in membranes (Toll and Snyder, 1982). What is not clear, however, is why mepyramine appears to be more potent as an antagonist of the recombinant rat H_1-receptor (expressed in C6 cells) than it is of the native H_1-receptor in rat brain membranes (table 4; Chang et al., 1979b; Hill and Young, 1980; Fujimoto et al., 1993). The recombinant study performed in rat C6 cells (Fujimoto et al., 1993) is complicated by the presence of a low level of endogenous H_1-receptors (Peakman and Hill, 1994), but a high affinity for mepyramine (K_D = 1 nM) has been deduced from functional studies in untransfected C6 cells (table 4; Peakman and Hill, 1994).

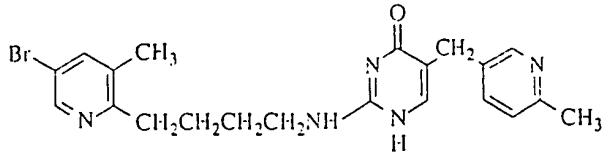
Site-directed mutagenesis has begun to shed some light on the binding domains for H_1-agonists and -antagonists. Amino acid sequence alignment of the cloned histamine H_1- and H_2-receptors (see fig. 5) has led to the suggestion that the third (TM3) and fifth (TM5) transmembrane domains of the receptor proteins are responsible for binding histamine (Birdsall, 1991; Timmerman, 1992a). Aspartate (107) in TM3 of the human H_1-receptor, which is conserved in all aminergic receptors, has



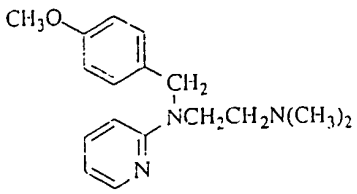
Doxepin (Z)-form†



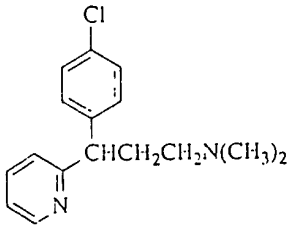
Triprolidine



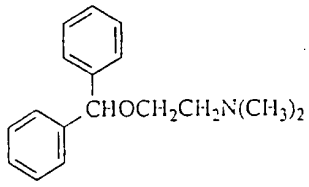
Temelastine



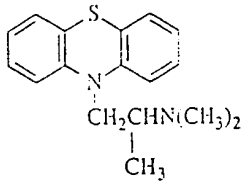
Mepyramine



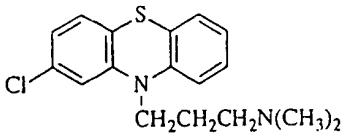
Chlorpheniramine



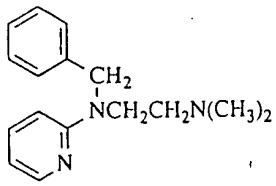
Diphenhydramine



Promethazine



Chlorpromazine



Tripelennamine

FIG. 3. Histamine H_1-receptor antagonists.

TABLE 4

Species variation in H_1-receptor antagonist potency (K_i, nM)

Antagonist	Guinea pig		Human		Rat		Bovine	
	h_1[a] (CHO)	H_1[b] (brain)	h_1 (CHO)	H_1 (brain)	h_1 (C6)	H_1 (brain)	h_1 (COS-7)	H1 (Adrenal Medulla)
Mepyramine	0.7	0.8	1.1, 4.0	1.0	1.7 (1.0)[c]	9.1	2.6	0.9
(+)-Chlorpheniramine	0.9	0.8	3.5, 2.5	4.2	7.5 (4.4)[c]	9.1	8.0	4.4
(−)-Chlorpheniramine	103	200	316	350	540 (>620)[c]	500	760	350
Triprolidine	0.7	0.2	1.0	3.7	2.0	5.6	n.d.	0.8

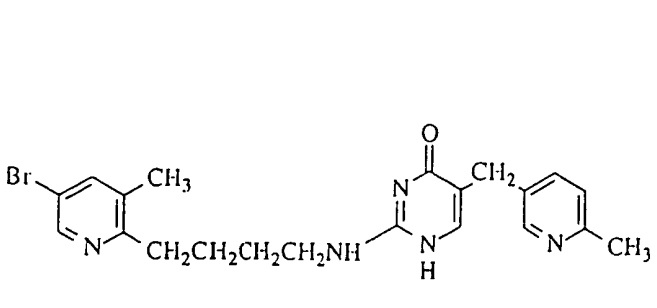
Unless otherwise stated, values show K_i determinations from inhibition of [^{3}H]mepyramine binding. n.d., not determined.

[a] h_1 = transfected H_1-receptor cDNA.

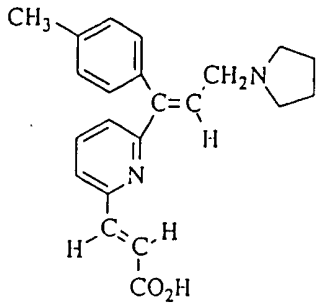
[b] H_1 = native/endogenous H_1-receptor.

[c] Values in parentheses show the values obtained from functional studies of the endogenous H_1-receptor present in rat C6 cells (Peakman & Hill, 1994).

been shown to be essential for the binding of histamine and H_1-receptor antagonists to the H_1-receptor (Ohta et al., 1994). In the α_2- and β_2-adrenoceptors, two serine residues in TM5 accept the phenolic hydroxyl groups of the catechol ring of noradrenaline. In the H_1-receptor, the residues corresponding to asparagine (198) and threonine (194) are in corresponding positions in TM5 of the human H_1-receptor. However, substitution of an alanine for threonine (194) did not influence either agonist or antagonist binding (Ohta et al., 1994; Moguilevsky et al., 1995). Substitution of alanine (198) for asparagine (198) substantially decreased agonist, but not antagonist affinity (Ohta et al., 1994; Moguilevsky et al., 1995). Similar mutations to the corresponding residues (threonine (203) and asparagine (207) in the guinea pig H_1-receptor sequence produce very similar results (Leurs et al., 1994a). It is interesting to note, however, that whereas 2-methylhistamine is similarly affected by the asparagine^{207} alanine mutation, the H_1-selective agonists 2-thiazolylethylamine, 2-pyridylethylamine, and 2-(3-bromophenyl)histamine are much less affected by this mutation (Leurs et al., 1994a). These data suggest



Temelastine



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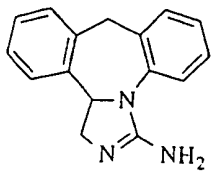

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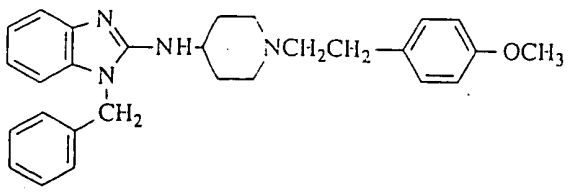
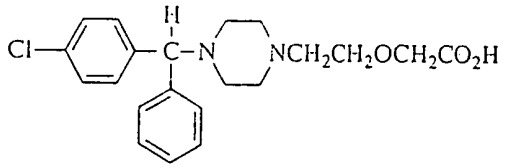
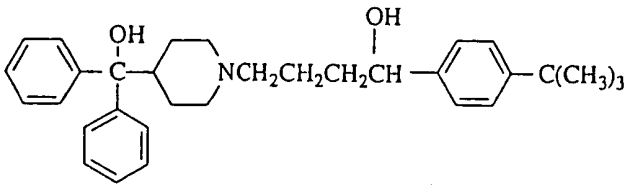


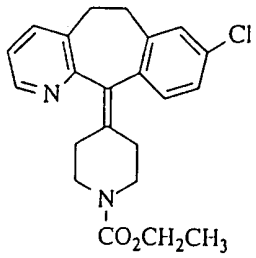
Astemizole



Cetirizine



Terfenadine



Loratidine

FIG. 4. "Nonsedating" H_1-receptor antagonists. †, The pharmaceutical product may be a mix of (E) and (Z) forms.

TABLE 5
"Nonsedating" H_1-receptor antagonists

Agent	pK_i value	Reference
Temelastine	9.5	Ter Laak et al. (1993)
Acrivastine	9.2	Leurs et al. (1995b)
Epinastine	8.9	Ter Laak et al. (1994)
Astemizole	8.3	Ter Laak et al. (1993)
Cetirizine	7.5	Ter Laak et al. (1993)
Terfenadine	7.1	Ter Laak et al. (1993)
Loratidine	6.8	Ter Laak et al. (1993)

Values are pK_i (−log dissociation constant) determined from inhibition of [^{3}H]mepyramine binding in homogenates of guinea pig cerebellum.

that asparagine (207) interacts with the N^{τ}-nitrogen of the imidazole ring of histamine. Furthermore, Leurs et al. (1995a) have recently shown that lysine (200) interacts with the N^{π}-nitrogen of histamine and is important for the activation of the H_1-receptor by histamine and the nonimidazole agonist, 2-pyridylethylamine. Interestingly, however, the lysine (200) alanine mutation did not alter the binding affinity of 2-pyridylethylamine to the guinea pig H_1-receptor (Leurs et al., 1995).

D. Signal Transduction Mechanisms

The primary mechanism by which histamine H_1-receptors produce functional responses in cells is the activation of phospholipase C via a pertussis toxin-insensitive G-protein that is probably related to the $G_{q/11}$ family of G-proteins (Hill, 1990; Leurs et al., 1995b). The number of tissues and cell types in which a histamine H_1-receptor–mediated increase in either inositol phosphate accumulation or intracellular calcium mobilization has been described is extensive, and further details are provided in several comprehensive reviews (Hill, 1990; Hill and Donaldson, 1992; Leurs et al., 1995b). Stimulation by histamine of [^{3}H]inositol phosphate accumulation and calcium mobilization has also been observed in Chinese hamster ovary (CHO) cells transfected with the human, bovine, and guinea pig H_1-receptor complementary deoxyribonucleic acid (cDNA) (Leurs et al., 1994c; Smit et al., 1996c; Iredale et al., 1993; Megson et al., 1995). It is worth noting, however, that in some tissues, histamine can stimulate inositol phospholipid hydrolysis independently of H_1-receptors. Thus, in the longitudinal smooth muscle of guinea pig ileum and neonatal

TABLE 6

Comparison of recombinant histamine receptors

Receptor	Species	Tissue of origin	Amino acid residues	Calculated mean weight (kDa)	Accession number	% Homology to human
H_1-receptor	Human	Genomic[a]	487	55.7	P35367	100
	Bovine	Adrenal medulla[b]	491	55.9	P30546	89.9
	Rat	Genomic[c]	486	55.6	P31390	87.8
	Guinea pig	Genomic[d]	488	55.6	P31389	82.9
	Mouse	Genomic[i]	489	55.6	D50095	84.0
H_2-receptor	Human	Genomic[e]	359	40.1	P25021	100
	Canine	Genomic[f]	359	40.2	P17124	92.5
	Rat	Genomic[g]	358	40.2	P25102	91.1
	Guinea pig	Liver[h]	359	40.5	JC4120	93.3
	Mouse	Genomic[j]	359	40.4	D50096	91.1

[a] De Backer et al. (1993)
[b] Yamashita et al. (1991b)
[c] Fujimoto et al. (1993)
[d] Horio et al. (1993)
[e] Gantz et al. (1991b)
[f] Gantz et al. (1991a)
[g] Ruat et al. (1991)
[h] Traiffort et al. (1995)
[i] Inove et al. (1996)
[j] Kobayashi et al. (1996)

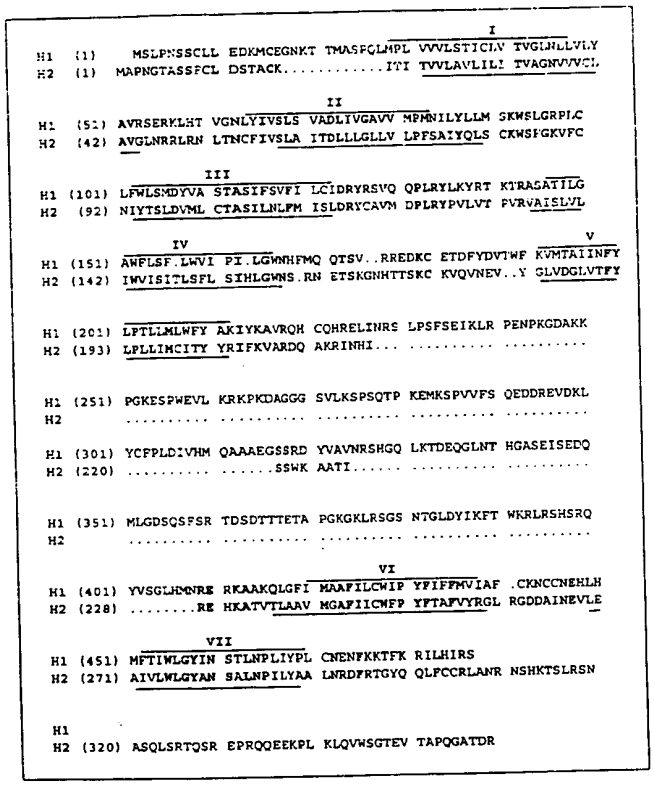


FIG. 5. Alignment of amino acid sequences of the human histamine H_1- and H_2-receptors. Residues that are identical in the two sequences are shown in bold. Lines show the putative transmembrane spanning domains.

rat brain (Donaldson and Hill, 1985, 1986b; Claro et al., 1987), a component can be identified in the response to histamine that is resistant to inhibition by H_1-receptor antagonists. It remains to be established, however, whether these effects are due to "tyramine-like" effects of histamine on neurotransmitter release (Bailey et al., 1987; Young et al., 1988a) or direct effects of histamine on the associated G-proteins (Seifert et al., 1994).

In addition to effects on the inositol phospholipid signaling systems, histamine H_1-receptor activation can lead to activation of several other signaling pathways, many of which appear to be secondary to changes in intracellular calcium concentration or the activation of protein kinase C. Thus, histamine can stimulate nitric oxide synthase activity (via a Ca^{2+}/calmodulin-dependent pathway) and subsequent activation of soluble guanylyl cyclase in a variety of different cell types (Schmidt et al., 1990; Leurs et al., 1991a; Yuan et al., 1993; Casale et al., 1985; Duncan et al., 1980; Hattori et al., 1990; Sertl et al., 1987). Arachidonic acid release and the synthesis of arachidonic acid metabolites such as prostacyclin and thromboxane A_2 can also be enhanced by H_1-receptor stimulation (Carter et al., 1988; Resink et al., 1987; Leurs et al., 1994c; Muriyama et al., 1990). Interestingly, in CHO-K1 cells transfected with the guinea pig H_1-receptor, the histamine-stimulated release of arachidonic acid is partially inhibited (approximately 40%) by pertussis toxin, whereas the same response in HeLa cells possessing a native H_1-receptor was resistant to pertussis toxin treatment (Leurs et al., 1994c). The reason for this difference remains to be established, but it does caution against the use of signal transduction pathways in highly expressed recombinant cell systems as a primary receptor classification tool. This point is best illustrated by the fact that in intact cellular systems, H_1-receptor activation can produce substantial changes in the intracellular levels of cAMP. In most tissues, histamine H_1-receptor activation does not activate adenylyl cyclase directly but acts to amplify direct cAMP responses to histamine H_2-, adenosine A_2-, and vasoactive intestinal polypeptide receptors (Palacios et al., 1978; Al-Gadi and Hill, 1987, 1985; Donaldson et al., 1989; Garbarg and Schwartz, 1988; Magistretti and Schorderet, 1985; Marley et al., 1991). In many of these cases, a role for both intracellular Ca^{2+} ions and protein kinase C has been implicated in this augmentation response (Al-Gadi and Hill, 1987; Schwabe et al., 1978; Garbarg and Schwartz, 1988). In CHO cells transfected with the bovine or guinea pig H_1-receptor, H_1-

receptor activation can also lead to both direct cAMP responses and to an enhancement of forskolin-stimulated cAMP formation (Leurs et al., 1994c; Sanderson et al., 1996).

III. Histamine H_2-Receptor

A. Distribution and Function

Unlike the situation with H_1-selective radioligands, attempts to map the distribution of H_2-receptors by using radiolabeled H_2-receptor antagonists have met with variable success (Hill, 1990). Thus, [^{3}H]cimetidine and [^{3}H]ranitidine have proved unsuitable as H_2-radioligands, and in the case of cimetidine, the binding to sites specifically labeled with the radioligand is potently inhibited by imidazoles that have very low H_2-receptor binding affinities (Burkard, 1978; Kendall et al., 1980; Smith et al., 1980; Bristow et al., 1981; Warrender et al., 1983). More success has been achieved with [^{3}H]tiotidine, which has a higher affinity for the H_2-receptor (table 7) in guinea pig brain, lung parenchyma, and CHO-K1 cells transfected with the human H_2-receptor cDNA (Gajtkowski et al., 1983; Norris et al., 1984; Sterk et al., 1986; Foreman et al., 1985a; Gantz et al., 1991a), although studies in rat brain were not successful (Maayani et al., 1982). At the present time, [^{125}I]iodoaminopotentidine is the most successful H_2-radioligand (Hirschfeld et al., 1992). It has high affinity (K_D = 0.3 nM) for the histamine H_2-receptor in brain membranes (Martinez-Mir et al., 1990; Ruat et al., 1990b; Traiffort et al., 1992a) and CHO-K1 cells expressing the cloned rat H_2-receptor (Traiffort et al., 1992b). The compound has also been used for autoradiographic mapping of H_2-receptors in mammalian brain (Ruat et al., 1990a; Traiffort et al., 1992a). In human brain, histamine H_2-receptors are widely distributed with highest densities (measured using [^{125}I]iodoaminopotentidine) in the basal ganglia, hippocampus, amygdala, and cerebral cortex (Traiffort et al., 1992a). Lowest densities were detected in cerebellum and hypothalamus (Traiffort et al., 1992a). A similar distribution has been observed in guinea pig brain (Ruat et al., 1990b). [^{125}I]Iodoazidopotentidine has successfully been used for irreversible labeling (Ruat et al., 1990b; Hirschfeld et al., 1992).

Most information to date on the distribution of histamine H_2-receptor, however, has been provided by functional studies in different tissues (Hill, 1990). Histamine H_2-receptor–stimulated cAMP accumulation or adenylyl cyclase activity has been demonstrated in a variety of tissues including brain (Hegstrand et al., 1976; Green et al., 1977; Kanof et al., 1977; Palacios et al., 1978; Gajtkowski et al., 1983; Al-Gadi and Hill, 1985, 1987), gastric cells (Soll and Wollin, 1979; Gespach et al., 1982), and cardiac tissue (Johnson et al., 1979a,b; Kanof and Greengard, 1979a; Johnson, 1982). Histamine H_2-receptors have a potent effect on gastric acid secretion, and the inhibition of this secretory process by H_2-receptor antagonists has provided evidence for an important physiological role of histamine in the regulation of gastric secretion (Black et al., 1972; Black and Shankley, 1985; Soll and Berglindh, 1987). High concentrations of histamine are also present in cardiac tissues of most animal species and can mediate positive chronotropic and inotropic effects on atrial or ventricular tissues via H_2-receptor stimulation (Black et al., 1972; Inui and Imamura, 1976; Levi et al., 1982; Hattori et al., 1983; Hattori and Levi, 1984; Hescheler et al., 1987; Levi and Alloatti, 1988). H_2-receptor–mediated smooth muscle relaxation has also been documented in airway, uterine, and vascular smooth muscle (Black et al., 1972; Reinhardt and Ritter, 1979; Gross et al., 1981; Eyre and Chand, 1982; Edvinsson et al., 1983; Foreman et al., 1985b; Ottosson et al., 1989). Finally, histamine H_2-receptors can inhibit a variety of functions within the immune system (Hill, 1990). H_2-receptors on basophils

TABLE 7
Histamine receptor radioligands

Receptor	Ligand	K_D	Tissue
H_1-receptor	[^{3}H]Mepyramine	0.8 nM	Guinea pig brain[a]
	[^{125}I]Iodobolpyramine	0.1 nM	Guinea pig brain[b]
	[^{125}I]Iodoazidophenpyramine	0.01 nM	Guinea pig cerebellum[c]
	[^{11}C]Mepyramine	1.0 nM	Human brain (in vivo)[d]
	[^{11}C]Doxepin	0.1 nM	Human brain (in vivo)[e]
H_2-receptor	[^{3}H]Tiotidine	25 nM	Guinea pig brain[f]
	[^{125}I]Iodoaminopotentidine	0.3 nM	Guinea pig brain[g]
	[^{125}I]Iodoazidopotentidine	10 nM	Guinea pig brain[g]
H_3-receptor	[^{3}H]R-(α)-methylhistamine	0.5 nM	Rat brain[h]
	[^{3}H]N$^\alpha$-methylhistamine	2.0 nM	Rat cerebral cortex[i]
	[^{125}I]Iodophenpropit	0.3 nM	Rat cerebral cortex[j]
	[^{125}I]Iodoproxyfan	0.065 nM	Rat striatum[k]
	[^{3}H]GR168320	0.1 nM	Rat cerebral cortex[l]

[a] Hill et al. (1981)
[b] Körner et al. (1986)
[c] Ruat et al. (1988)
[d] Villemagne et al. (1991)
[e] Yanai et al. (1995)
[f] Gajtkowski et al. (1983)
[g] Ruat et al. (1990a)
[h] Arrang et al. (1990)
[i] Clark and Hill (1995)
[j] Jansen et al. (1992)
[k] Ligneau et al. (1994)
[l] Brown et al. (1994)

and mast cells have been shown to negatively regulate the release of histamine (Bourne et al., 1971; Lichtenstein and Gillespie, 1975; Lett-Brown and Leonard, 1977; Ting et al., 1980; Plaut and Lichtenstein, 1982). Furthermore, there is increasing evidence that H_2-receptors on lymphocytes can inhibit antibody synthesis, T-cell proliferation, cell-mediated cytolysis, and cytokine production (Bourne et al., 1971; Melmon et al., 1974, 1981; Griswold et al., 1984; Khan et al., 1985, 1986; Sansoni et al., 1985; Melmon and Khan, 1987). In the CNS, histamine H_2-receptor activation can inhibit nerve cells (Haas and Bucher, 1975; Haas and Wolf, 1977), but the most intriguing action is a block of the long-lasting after-hyperpolarization and the accommodation of firing, an effect with a remarkably long duration leading to potentiation of excitation in rodents (Haas and Konnerth, 1983; Haas and Greene, 1986) and human brain (Haas et al., 1988). A slow excitation is also common (Greene and Haas, 1989; Phelan et al., 1990). Synaptic transmission in the hippocampus is profoundly enhanced (Kostopoulos et al., 1988), and synaptic plasticity is induced or enhanced (Brown et al., 1995). An increase of the hyperpolarization-activated current has also been described in thalamic relay neurons (McCormick and Williamson, 1991). Indications for non-cAMP mediated actions of H_2-receptor activation are given by Haas et al. (1978) and Jahn et al. (1995).

B. H_2-Selective Ligands

The initial definition of the H_1- and H_2-subclasses of histamine receptor by Ash and Schild (1966) and Black and colleagues (1972) led to a successful search for H_2-receptor selective antagonists with clinical relevance for the treatment of peptic ulcer. Burimamide was the first compound developed that showed selectivity for the H_2-receptor (Black et al., 1972), but more recent work has shown that this compound is a more potent H_3-receptor antagonist (Arrang et al., 1983). Cimetidine and metiamide were developed directly from burimamide (Black et al., 1974; Brimblecombe et al., 1975; Ganellin, 1978). Since then, a large number of compounds have been developed with H_2-receptor antagonist properties [see Ganellin (1992) for review]. These include ranitidine (Bradshaw et al., 1979), tiotidine (Yellin et al., 1979), nizatidine (Lin et al., 1986), famotidine (Takeda et al., 1982), and mifentidine (Donetti et al., 1984), which have been extensively used for characterization purposes (table 3; fig. 6). Iodoaminopotentidine (K_D = 2.5 nM) is one of the most potent H_2-receptor antagonists available, and, as mentioned above, this compound has been used as a successful radioligand (Hirschfeld et al., 1992). Most H_2-receptor antagonists are polar compounds and penetrate poorly into the CNS. Although this property is of great use for selective actions on peripheral tissues (e.g., gastric mucosa), it does limit the use of the com-

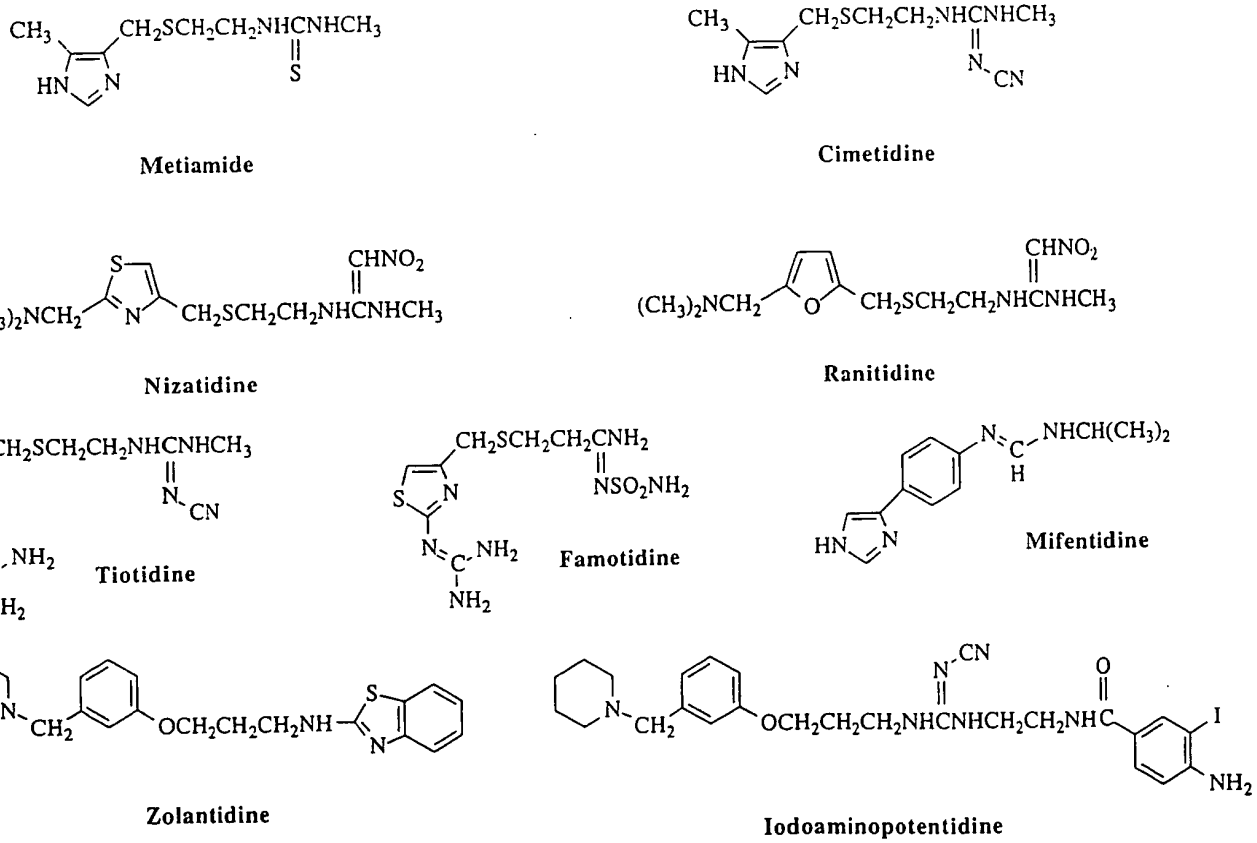


FIG. 6. Histamine H_2-receptor antagonists.

pounds for the in vivo evaluation of H_2-receptor function within the CNS. However, one compound (zolantidine) is a potent and selective brain-penetrating histamine H_2-receptor antagonist (table 3; Calcutt et al., 1988; Young et al., 1988b). Both cimetidine and ranitidine have been shown to demonstrate inverse agonism on histamine H_2-receptors transfected into CHO cells (Smit et al., 1996a). Thus, in CHO cells expressing high levels of H_2-receptors, in which a considerable constitutive activation of H_2-receptors was demonstrated, cimetidine and ranitidine inhibited basal adenylyl cyclase activity (Smit et al., 1996a). In contrast, burimamide behaved as a neutral antagonist (Smit et al., 1996a).

4-Methylhistamine was the first agonist described that had any selectivity for the H_2-receptor (Black et al., 1972), although more potent and selective H_2-agonists are now available (table 2). It is noteworthy that many of the selective H_2-agonists exhibit H_1- or H_3-antagonist properties (see table 2); consequently the demonstration of H_2-agonism in a given tissue or cell type needs confirming with H_2-antagonists. Impromidine is approximately 48 times more potent than histamine in mediating atrial chronotropic responses, but in several other H_2-receptor–containing tissues, its relative potency and efficacy are lower (Durant et al., 1978; Leurs et al., 1995b). A large number of impromidine analogues have been synthesized and evaluated for H_2-agonism. These studies have led to the development of the potent H_2-agonists, sopromidine and arpromidine (table 2; Timmerman, 1992c). Arpromidine and analogues are potential candidates for treatment of congestive heart failure (Buschauer, 1989; Buschauer and Baumann, 1991; Mörsdorf et al., 1990). Another potent H_2-agonist has been derived as an analogue of dimaprit by considering cyclic forms of the isothiourea group (Eriks et al., 1992).

C. Receptor Structure

Photoaffinity binding studies using [^{125}I]iodoazidopotentidine and sodium dodecyl sulfate-polyacrylamide gel electrophoresis have suggested that the H_2-receptor in guinea pig hippocampus and striatum has a molecular weight of 59 kDa (Ruat et al., 1990b). However, comparison with the calculated molecular weights (40.2 to 40.5 kDa) for the recently cloned H_2-receptors (table 6) suggests that the native H_2-receptor in guinea pig brain is glycosylated. Consistent with this proposal, it is noteworthy that all of the cloned H_2-receptor proteins possess N-glycosylation sites in the N-terminus region (Gantz et al., 1991a,b; Ruat et al., 1991; Traiffort et al., 1995). Removal of these glycosylation sites by site-directed mutagenesis, however, has shown that N-glycosylation of the H_2-receptor is not essential for cell surface localization, ligand binding, or coupling via G_s to adenylyl cyclase (Fukushima et al., 1995).

The H_2-receptor was first cloned by Gantz and colleagues using the polymerase chain reaction to amplify a partial length H_2-receptor sequence from canine gastric parietal cDNA using degenerate oligonucleotide primers (Gantz et al., 1991b). This sequence was then used to identify a full length H_2-receptor clone following screening of a canine genomic library (Gantz et al., 1991b). Rapid cloning of the rat, human, guinea pig, and mouse H_2-receptors followed (Gantz et al., 1991a; Ruat et al., 1991; Traiffort et al., 1995; Kobayashi et al., 1996). These DNA sequences encode for a 359 (canine, human, guinea pig) or 358 (rat) receptor protein that has the general characteristics of a G-protein-coupled receptor. The most notable difference between the structure of the cloned H_2- and H_1- receptors is the much shorter 3rd intracellular loop of the H_2-receptor and the longer H_2-receptor C terminus. Expression of the rat and human H_2-receptor proteins in CHO cells has revealed the expected pharmacological specificity of H_2-receptors as judged by radioligand binding studies using [^{125}I]iodoaminopotentidine (Traiffort et al., 1992b; Leurs et al., 1994c). Recent chromosomal mapping studies have assigned the H_2-receptor gene to human chromosome 5 (Traiffort et al., 1995).

Comparison of the H_2-receptor sequence with other biogenic amine G-protein–coupled receptors has indicated that an aspartate in TM3 and an aspartate and threonine residue in TM5 are responsible for binding histamine (Birdsall, 1991). Replacement of aspartate (98) by an asparagine residue in the canine H_2-receptor results in a receptor that does not bind the antagonist tiotidine and does not stimulate cAMP accumulation in response to histamine (Gantz et al., 1992). Similarly, changing the aspirate (186) of TM5 to an alanine resulted in complete loss of tiotidine binding without affecting the EC_{50} for histamine-stimulated cAMP formation (Gantz et al., 1992). Changing the threonine (190) to an alanine, however, resulted in a lower K_D for tiotidine and a reduction in both the maximal cAMP response and histamine EC_{50} value (Gantz et al., 1992). Mutation of Asp (186) and Gly (187) in the canine H_2-receptor (to Ala (186) and Ser (187), however, produces a bifunctional receptor that can be stimulated by adrenaline and inhibited by both propranolol and cimetidine (Delvalle et al., 1995). Thus, these data suggest that the pharmacological specificity of the H_2-receptor resides in only a few key amino acid residues.

Other site-directed mutagenesis studies on the H_2-receptor have been very limited. However, Smit et al. (1996) have identified a residue in the second intracellular loop [leucine (124)] of the rat H_2-receptor, which appears necessary for efficient coupling to G_s.

D. Signal Transduction Mechanisms

It is generally accepted that histamine H_2-receptors couple to adenylyl cyclase via the GTP-binding protein G_s (Johnson, 1982; Hill, 1990; Leurs et al., 1995b). Histamine is a potent stimulant of cAMP accumulation in many cell types (Johnson, 1982), particularly those of CNS origin (Daly, 1977). Thus, H_2-receptor–mediated effects on cAMP accumulation have been observed in

brain slices (Al-Gadi and Hill, 1985; Palacios et al., 1978), gastric mucosa (Soll and Wollin, 1979; Chew et al., 1980; Batzri et al., 1982; Gespach et al., 1982), fat cells (Grund et al., 1975; Keller et al., 1981), cardiac myocytes (Warbanow and Wollenberger, 1979), vascular smooth muscle (Reinhardt and Ritter, 1979), basophils (Lichtenstein and Gillespie, 1975), and neutrophils (Busse and Sosman, 1977). Furthermore, H_2-receptor–mediated cAMP accumulation has been demonstrated in CHO cells transfected with the rat, canine, or human H_2-receptor cDNA (Gantz et al., 1991a,b; Leurs et al., 1994b; Fukushima et al., 1995).

Direct stimulation of adenylyl cyclase activity in cell-free preparations has been detected in both brain and cardiac muscle membranes (Hegstrand et al., 1976; Green et al., 1977; Green and Maayani, 1977; Kanof et al., 1977; Johnson et al., 1979a,b; Kanof and Greengard, 1979a,b; Newton et al., 1982; Olianas et al., 1984). However, caution is required regarding the interpretation of receptor characterization studies using histamine-stimulated adenylyl cyclase activity alone (Hill, 1990). A striking feature of studies of histamine H_2-receptor–stimulated adenylyl cyclase activity in membrane preparations is the potent antagonism observed with certain neuroleptics and antidepressants (table 8; Spiker et al., 1976; Green et al., 1977; Green and Maayani, 1977; Kanof and Greengard, 1978; Green, 1983). It is notable, however, that most of the neuroleptics and antidepressants are approximately 2 orders of magnitude weaker as antagonists of histamine-stimulated cAMP accumulation in intact cellular systems (table 8; Tuong et al., 1980; Kamba and Richelson, 1983; Hill, 1990). One potential explanation of these differences resides within the buffer systems used for the cell-free adenylyl cyclase assays. Some differences in potency of some antidepressants and neuroleptics have been observed when membrane binding of H_2-receptors has been evaluated using [^{125}I]iodoaminopotentidine (table 8; Traiffort et al., 1991). However, invariably the differences observed in the K_i values deduced from ligand binding studies in different buffers are not as large as the differences in K_B values obtained from functional studies (table 8). For example, in the case of amitriptyline, no difference was observed in binding affinity in Krebs and Tris buffers (Traiffort et al., 1991).

In addition to G_s-coupling to adenylyl cyclase, there are reports of H_2-receptors coupling to other signaling systems. For example, in gastric parietal cells. H_2-receptor stimulation has been shown to increase the intracellular free concentration of calcium ions (Chew, 1985. 1986; Chew and Petropoulos, 1991; Malinowska et al., 1988; Delvalle et al., 1992a). A similar calcium response to histamine H_2-receptor stimulation has also been observed in HL-60 cells (Mitsuhashi et al., 1989; Seifert et al., 1992) and in hepatoma-derived cells transfected with the canine H_2-receptor cDNA (Delvalle et al., 1992b). In these latter cells, the influence on $[Ca^{2-}]_i$ was accompanied by both an increase in inositol trisphosphate accumulation and a stimulation of cAMP accumulation (Delvalle et al., 1992b). Interestingly, the H_2-receptor–stimulated calcium and inositol trisphosphate responses in these cells were both inhibited by cholera toxin treatment (but not by pertussis toxin), whereas cholera toxin produced the expected increase in cAMP levels (Delvalle et al., 1992a,b). In single parietal cells, H_2-receptors have been shown to release calcium from intracellular calcium stores (Negulescu and Machen, 1988). It should be noted, however, that no effect of H_2-agonists was observed on inositol phosphate accumulation or intracellular calcium levels in CHO cells transfected with the human H_2-receptor (Leurs et al., 1994a).

TABLE 8

Comparison of antagonist K_B values for inhibition of H_2-receptor-stimulated adenylyl cyclase activity in membranes and cyclic AMP accumulation in intact cellular systems

Antagonist	Antagonist K_B value (μM)			Binding studies (K_i, μM)	
	Slices[a]	Dissociated cells[b]	Homogenates[c,d,e]	Krebs buffer	Tris buffer
Cimetidine	0.6	0.5	0.9		
Metiamide	0.8	n.d.	1.0		
Tiotidine	n.d.	0.03	0.03	0.02	0.007
Cyproheptadine	5.7	n.d.	0.04		
Mianserin	10.0	2.8	0.07	1.01	0.20
Imipramine	>10	3.3	0.2		
Amitriptyline	3.5	1.9	0.05	0.09	0.09
Chlorpromazine	5.9	3.0	0.04		
Haloperidol	>10	29	0.08	1.61	0.42

Measurements were made of H_2-mediated adenylyl cyclase activity in homogenates of guinea pig hippocampus, impromidine-stimulated cyclic AMP accumulation in guinea pig hippocampal slices, and of H_2-mediated cyclic AMP accumulation in dissociated hippocampal tissue. n.d., not determined.

[a] Tuong et al. (1980)
[b] Kamba et al. (1983)
[c] Green et al. (1977)
[d] Kanof and Greengard (1978)
[e] Kanof and Greengard (1979a,b)
[f] Traiffort et al. (1991)

Thus, the effect of H_2-receptor stimulation on intracellular calcium signaling may be very cell-specific.

In CHO cells transfected with the rat H_2-receptor, H_2-receptor stimulation produces both an increase in cAMP accumulation and an inhibition of P_{2u}-receptor–mediated arachidonic acid release (Traiffort et al., 1992b). Interestingly, however, the effect on phospholipase A_2 activity (i.e., arachidonic acid release) was not mimicked by forskolin, PGE_1, or 8-bromo-cAMP, suggesting a mechanism of activation that is independent of cAMP-mediated protein kinase A activity (Traiffort et al., 1992b). However, in CHO cells transfected with the human H_2-receptor, no inhibitory effects of H_2-receptor stimulation were observed on phospholipase A_2 activity (Leurs et al., 1994b). This observation suggests that these cAMP-independent effects might depend on the level of receptor expression or subtle differences between clonal cell lines.

IV. Histamine H_3-Receptor

A. Distribution and Function

The high apparent affinity of R-(α)-methylhistamine for the histamine H_3-receptor has enabled the use of this compound as a radiolabeled probe (Arrang et al., 1987). This compound has been successfully used to identify a single binding site in rat cerebral cortical membranes, which in phosphate buffer has the pharmacological characteristics of the H_3-receptor (Arrang et al., 1987, 1990). [^{3}H]R-(α)-methylhistamine binds with high affinity (K_D = 0.3 nM) to rat brain membranes, although the binding capacity is generally low (approximately 30 fmol/mg protein; (Arrang et al., 1987). Autoradiographic studies with [^{3}H]R-(α)-methylhistamine have demonstrated the presence of specific thioperamide-inhibitable binding in several rat brain regions, particularly cerebral cortex, striatum, hippocampus, olfactory nucleus, and the bed nuclei of the stria terminalis, which receive ascending histaminergic projections from the magnocellular nuclei of the posterior hypothalamus (Arrang et al., 1987; Pollard et al., 1993). H_3-receptors have also been visualized in human brain and the brain of nonhuman primates (Martinez-Mir et al., 1990). H_3-receptor binding has been additionally characterized using [^{3}H]R-(α)-methylhistamine in guinea pig cerebral cortical membranes (Kilpatrick and Michel, 1991), guinea pig lung (Arrang et al., 1987), guinea pig intestine, and guinea pig pancreas (Korte et al., 1990). N$^\alpha$-methylhistamine has also proved successful as a radiolabeled probe for the H_3-receptor. Although the relative agonist activity of N$^\alpha$-methylhistamine (with respect to histamine) is fairly similar for all three histamine receptor subtypes (table 2), the binding affinity of histamine and N$^\alpha$-methylhistamine for the H_3-receptor is several orders of magnitude higher than for either the H_1- or H_2-receptors (Hill et al., 1977; Ruat et al., 1990b). This ligand can identify high-affinity H_3-receptor sites in both guinea pig (Korte et al., 1990) and rat (West et al., 1990; Kathman et al., 1993; Clark and Hill, 1995) brain.

The binding of ^{3}H-agonists to H_3-receptors in brain tissues has been shown to be regulated by guanine nucleotides, implying a linkage to heterotrimeric G-proteins (Arrang et al., 1987, 1990; Zweig et al., 1992; Clark and Hill, 1995). The binding of H_3-receptor agonists also seems to be sensitive to several cations. Magnesium and sodium ions have been shown to inhibit [^{3}H]R-(α)-methylhistamine binding in rat and guinea pig brain (Kilpatrick and Michel, 1991), and the presence of calcium ions has been reported to reveal heterogeneity of agonist binding (Arrang et al., 1990). The inhibitory effect of sodium ions on agonist binding means that higher B_{max} values are usually obtained in sodium-free Tris buffers compared with that in Na/K phosphate buffers (Clark and Hill, 1995). West et al. (1990) have suggested that multiple histamine H_3-receptor subtypes exist in rat brain (termed H_{3A} and H_{3B}) on the basis of [^{3}H]N$^\alpha$-methylhistamine binding in rat cerebral cortical membranes in 50 mM Tris buffer. Under these conditions, the selective H_3-antagonist thioperamide can discriminate two affinity binding states (West et al., 1990). However, Clark and Hill (1995) have noted that the observed heterogeneity of thioperamide binding is dependent on the concentration of sodium ions or guanine nucleotides within the incubation medium. Thus, in the presence of 100 mM sodium chloride, thioperamide binding conforms to a single binding isotherm (Clark and Hill, 1995). The simplest interpretation of these data is that the H_3-receptor can exist in different conformations for which thioperamide, but not agonists or other H_3-antagonists (e.g., clobenpropit), can discriminate. Clark and Hill (1995) have suggested that the equilibrium between these conformations is altered by guanine nucleotides or sodium ions. If this hypothesis is correct, it is likely that the different binding sites represented resting, active, or G-protein–coupled conformations of the H_3-receptor. Furthermore, if thioperamide preferentially binds to uncoupled receptors, then this compound should exhibit negative efficacy in functional assays.

More recently, radiolabeled H_3-receptor antagonists have become available. The first compound to be developed was [^{125}I]iodophenpropit, which has been used to successfully label H_3-receptors in rat brain membranes (Jansen et al., 1992). Inhibition curves for thioperamide and iodophenpropit were consistent with interaction with a single binding site, but H_3-receptor agonists were able to discriminate high- [4 nM for R-(α)-methylhistamine] and low- [0.2 μM for R-(α)-methylhistamine] affinity binding sites (Jansen et al., 1992). More recently, [^{3}H]GR16820 (Brown et al., 1994) and [^{125}I]iodoproxyfan (Ligneau et al., 1994) have also proved useful as high-affinity radiolabeled H_3-antagonists. [^{125}I]iodoproxyfan (Stark et al., 1996a) is the most potent and selective ligand available at the present time with a K_D of 65 pM (Ligneau et al., 1994). In rat striatum, in the

presence of guanine nucleotides such as guanosine 5′*O*-(3-thiotriphosphate) (GTPγS), 40% of the binding sites exhibited a 40-fold lower affinity for H_3-agonists, providing further evidence for a potential linkage of H_3-receptors to G-proteins (Ligneau et al., 1994). [^{3}H]thioperamide and [^{3}H]5-methylthioperamide have also been used to label H_3-receptors in rat brain membranes (Alves-Rodrigues et al., 1996; Yanai et al., 1994). However, [^{3}H]thioperamide was shown to bind additionally to low-affinity, high-capacity, non H_3-receptor sites in this tissue (Alves-Rodrigues et al., 1996).

In addition to data obtained from ligand binding studies, evidence for the localization of histamine H_3-receptors has also come from functional studies, primarily involving inhibition of neurotransmitter release. The H_3-receptor was first characterized as an autoreceptor-regulating histamine synthesis and release from rat cerebral cortex, striatum, and hippocampus (Arrang et al., 1983, 1985b,c 1987a, 1988a,b). H_3-receptor–mediated inhibition of histamine release has also been observed in human cerebral cortex (Arrang et al., 1988a). Differences in the distribution of H_3-receptor binding sites and the levels of histidine decarboxylase (an index of histaminergic nerve terminals) suggested at an early stage that H_3-receptors were not confined to histamine-containing neurons within the mammalian CNS (Arrang et al., 1987; Van der Werf and Timmerman, 1989). This has been confirmed by the observations that H_3-receptors can regulate serotonergic (Schlicker et al., 1988), noradrenergic (Schlicker et al., 1989, 1992), cholinergic (Clapham and Kilpatrick, 1992), and dopaminergic (Schlicker et al., 1993) neurotransmitter release in mammalian brain. Histamine H_3-receptor activation inhibits the firing of the histamine-neurons in the posterior hypothalamus through a mechanism different from autoreceptor functions found on other aminergic nuclei, presumably a block of Ca^{2+}-current (Haas, 1992). Electrophysiological evidence for reduction of excitatory transmitter release (glutamate) has been presented by Brown and Reymann (unpublished data, 1996).

Inhibitory effects of H_3-receptor activation on neurotransmission have also been documented in the periphery. Thus, H_3-receptors have been identified regulating the release of sympathetic neurotransmitters in guinea pig mesenteric artery (Ishikawa and Sperelakis, 1987), human saphenous vein (Molderings et al., 1992), guinea pig atria (Endou et al., 1994; Imamura et al., 1994), and human heart (Imamura et al., 1995). Inhibition of parasympathetic nerve activity has also been observed in guinea pig ileum and human bronchi and trachealis (Trzeciakowski, 1987; Tamura et al., 1988; Ichinose et al., 1989; Ichinose and Barnes, 1989; Hew et al., 1990; Menkveld and Timmerman, 1990; Leurs et al., 1991a,b; Poli et al., 1991). An inhibitory effect of H_3-receptor stimulation on release of neuropeptides (tachykinins or calcitonin gene-related peptide) from sensory C fibers has been reported from airways (Ichinose et al., 1990), meninges (Matsubara et al., 1992), skin (Ohkubo and Shibata, 1995), and heart (Imamura et al., 1996). A modulation of acetylcholine, capsaicin, and substance P effects by histamine H_3-receptors in isolated perfused rabbit lungs has also been reported (Delaunois et al., 1995).

There is evidence that H_3-receptor stimulation can inhibit the release of neurotransmitters from nonadrenergic-noncholinergic nerves in guinea pig bronchioles (Burgaud and Oudart, 1994) and ileum (Taylor and Kilpatrick, 1992). Interestingly, in guinea pig ileum, the H_3-antagonists betahistine and phenylbutanoylhistamine were much less potent as inhibitors of H_3-mediated effects on nonadrenergic-noncholinergic transmission than they were as antagonists of histamine release in rat cerebral cortex (Taylor and Kilpatrick, 1992). A similar low potency has been observed for these two antagonists for antagonism of H_3-receptor–mediated [^{3}H]acetylcholine release from rat entorhinal cortex (Clapham and Kilpatrick, 1992) and antagonism of H_3-receptor–mediated 5-hydroxytryptamine (5-HT) release from porcine enterochromaffin cells (Schworer et al., 1994). These observations provide support for the possible existence of distinct H_3-receptor subtypes, but these responses need to be investigated further to exclude alternative explanations. For example, Arrang et al. (1995) have recently shown that phenylbutanoylhistamine can inhibit [^{3}H]acetylcholine release from rat entorhinal cortex slices and synaptosomes via a nonhistamine receptor mechanism. Thus, the potency of phenylbutanoylhistamine as an H_3-receptor antagonist in these preparations may be greatly underestimated because of the additional nonspecific properties of the drug (Arrang et al., 1995).

The observed inhibitory effect of H_3-receptor stimulation on 5-HT release from porcine enterochromaffin cells in strips of small intestine (Schworer et al., 1994) provides evidence for H_3-receptors regulating secretory mechanisms in nonneuronal cells. This observation suggests that H_3-receptors may also be present in gastric mast cells or enterochromaffin cells and exert an inhibitory influence on histamine release and gastric acid secretion. Consistent with this suggestion, H_3-receptor activation has been shown to inhibit gastric acid secretion in conscious dogs (Soldani et al., 1993). An autoregulation of histamine synthesis by histamine H_3-receptors has also been reported in isolated rabbit fundic mucosal cells (Hollande et al., 1993).

H_3-receptors have been shown to relax rabbit middle cerebral artery via an endothelium-dependent mechanism involving both nitric oxide and prostanoid release (Ea Kim and Oudart, 1988; Ea Kim et al., 1992). Finally, there is a report that H_3-receptor activation can stimulate adrenocorticotropic hormone release from the pituitary cell line AtT-20 (Clark et al., 1992)

B. H_3-Receptor Selective Ligands

The initial characterization of the H_3-receptor made use of the relative high affinity of the agonists N$^{\alpha}$-methylhistamine and histamine for the H_3-receptor compared with the H_1- and H_2-receptors together with the H_3-antagonist properties of impromidine (H_2-agonist), burimamide (H_2-antagonist), and betahistine (H_1-agonist) (Arrang et al., 1983, 1985a). Since then, several selective ligands (both agonists and antagonists) have been developed that show little effect on H_1- and H_2-receptors. The first selective H_3-agonist was R-(α)-methylhistamine (fig. 2), which capitalized on the marked stereoselectivity of agonist binding to the H_3-receptor compared with that to the other histamine receptors (Arrang et al., 1985c). Thus, R-(α)-methylhistamine is two orders of magnitude more potent as an H_3-agonist than the corresponding S-isomer (table 2). R-α_1S-β-dimethylhistamine showed slightly higher potency and even higher selectivity (Lipp et al., 1992). Imetit [S-[2–4(5)-imidazolylethylisothiourea] is a highly selective, full H_3-agonist that appears to be more potent than R-(α)-methylhistamine (table 2; Garbarg et al., 1992; Howson et al., 1992; Van der Goot et al., 1992). Both R-(α)-methylhistamine and imetit have been shown to be active in vivo at low doses (Arrang et al., 1987a; Garbarg et al., 1992). Azomethine derivatives of R-(α)-methylhistamine were prepared as lipophilic prodrugs to improve the bioavailability of the hydrophilic drug, particularly its entry into the brain (Krause et al., 1995). Immepip is another potent H_3-agonist that has been developed from histamine by extending the alkyl side chain to four methylene groups and incorporating the amino function within a piperidine ring (table 2; Vollinga et al., 1994). Most recently, the H_3-agonist potency of a cyclic, conformationally restricted analogue of histamine (immepyr) has been reported (Shih et al., 1995). This compound has been resolved and the (+)-immepyr shown to have an H_3-binding affinity (K_i = 2.8 nM) one order of magnitude higher than the corresponding (-)-isomer (Shih et al., 1995). In guinea pig ileum, however, (+)-immepyr was one order of magnitude less potent (pD_2 7.1) than R-(α)-methylhistamine (pD_2 8.2) as an H_3-agonist (Shih et al., 1995).

Thioperamide was the first potent and selective H_3-receptor antagonist to be described (Arrang et al., 1987). This compound appears to act as a competitive antagonist in most functional assays of H_3-receptor activity (Arrang et al., 1987; Hew et al., 1990; Menkveld and Timmerman, 1990), although Clark and Hill (1995) have suggested that it may possess inverse agonist properties. More recently, several other potent H_3-antagonists have been described (table 3; fig. 7), including clobenpropit (Kathman et al., 1993), iodophenpropit (Jansen et al., 1992), GR175737 (Clitherow et al., 1996), iodoproxyfan (Ligneau et al., 1994; Schlicker et al., 1996), impentamine (Vollinga et al., 1995; Leurs et al., 1996), ethers (Ganellin et al., 1996; Stark et al., 1996a), and carbamates (Stark et al., 1996b). These compounds have initiated some further discussion regarding potential H_3-receptor subtypes. Thus, iodoproxyfan behaves as a partial agonist in both guinea pig ileum and mouse cerebral cortical slices, whereas its noniodinated analogue only exhibits slight agonist activity in the mouse brain preparation (Schlicker et al., 1996). In guinea pig ileum, the noniodinated analogue of iodoproxyfan is a pure antagonist (pA_2 7.12; Schlicker et al., 1996). These observations point to differences in receptor structure in the two preparations (perhaps species homologues?), but they could equally well be accommodated by differences in the efficiency of H_3-receptor–effector coupling between the two tissues. A similar observation has been made with a series of homologues of histamine in which the ethylene side chain was modified (Leurs et al., 1996). Lengthening the side chain of histamine from two to five methylene groups results in the highly selective H_3-antagonist impentamine, which is equipotent with thioperamide as a competitive antagonist in guinea pig jejunum (table 3; Vollinga et al., 1995). However, in mouse brain cerebral cortical slices, impentamine (like iodoproxyfan) exhibits partial agonist activity (Leurs et al., 1996). At the present time, differences in receptor-effector coupling (and hence H_3-receptor reserve) between mouse brain and guinea pig small intestine provide the simplest explanation for these observations.

Although many of the H_3-selective ligands have been fully characterized in terms of selectivity for each of the three histamine receptors, it is worth stressing that the evaluation of H_3-receptor ligands against other receptor systems is more limited. This needs to be borne in mind, particularly, when considering the in vivo use of these compounds. For example, iodophenpropit (K_i 11 nM) and thioperamide (K_i 120 nM) have both been shown to interact with 5-HT_3-receptors (Leurs et al., 1995c), whereas iodoproxyfan did not (Schlicker et al., 1995).

C. Receptor Structure

Structural information on the histamine H_3-receptor is very limited, primarily because of a lack of success in cloning the H_3-receptor cDNA. At the present time, there are only two reports of H_3-receptor purification studies. Using [^{3}H]histamine as a radioligand, Zweig et al. (1992) have reported the solubilization of an H_3-receptor protein from bovine whole brain. Size-exclusion chromatography has revealed an apparent molecular mass of 220 kDa (Zweig et al., 1992). However, because the solubilized receptor retained its guanine nucleotide sensitivity, it is likely that the molecular mass of 220 kDa represents a complex of receptor, G-protein, and digitonin (Zweig et al., 1992). Cherifi et al. (1992) have reported the solubilization (with Triton X-100) and purification of the H_3-receptor protein from the human gastric tumoral cell line HGT-1. After gel filtration and sepharose-thioperamide affinity chromatography, protein has been purified with a molecular mass of approx-

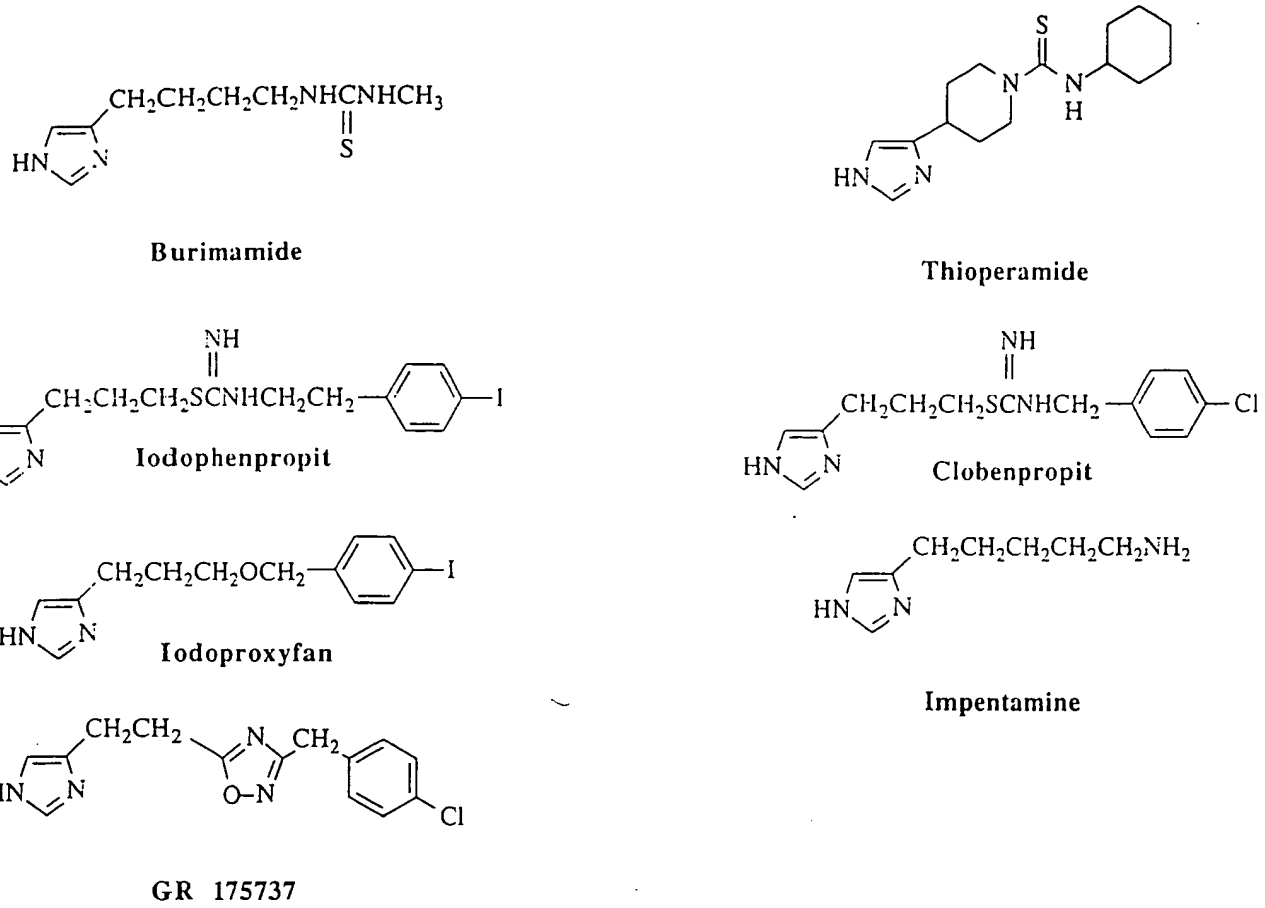


FIG. 7. Histamine H_3-receptor antagonists.

imately 70 kDa (Cherifi et al., 1992). However, it remains to be established whether this protein is the histamine H_3-receptor.

D. Signal Transduction Mechanisms

The signal transduction pathways used by the histamine H_3-receptor remain largely subject to speculation, but there is increasing evidence to suggest that this receptor belongs to the superfamily of G-protein–coupled receptors. Evidence for this has largely been obtained from ligand-binding studies involving the modulation by guanine nucleotides of H_3-agonist binding (Arrang et al., 1990; West et al., 1990; Kilpatrick and Michel, 1991; Zweig et al., 1992; Clark and Hill, 1995) and of H_3-agonist inhibition of ^{3}H-antagonist binding (Jansen et al., 1992, 1994; Ligneau et al., 1994). The most direct evidence for a functional H_3-receptor–G-protein linkage has come from studies of [^{35}S]GTPγS binding to rat cerebral cortical membranes (Clark and Hill, 1996). In the presence of H_1- and H_2-receptor antagonists (0.1 μM mepyramine and 10 μM tiotidine), both R-α-methylhistamine and N^{α}-methylhistamine produced a concentration-dependent stimulation of [^{35}S]GTPγS binding (EC_{50} = 0.4 and 0.2 nM, respectively) in rat cerebral cortical membranes (Clark and Hill, 1996). Furthermore, this response was abolished by pretreatment of membranes with pertussis toxin, implying a direct coupling to a G_i or G_o protein (Clark and Hill, 1996). Evidence for an involvement of pertussis toxin-sensitive G-proteins in the response to H_3-receptor stimulation has also come from studies of histamine H_3-receptor signaling in human and guinea pig heart (Endou et al., 1994; Imamura et al., 1995). In these tissues, histamine H_3-receptor–stimulation seems to lead to an inhibition of N-type Ca^{2+} channels responsible for voltage-dependent release of noradrenaline (Endou et al., 1994; Imamura et al., 1995).

Very little is known about the intracellular signal transduction pathways initiated by histamine H_3-receptor activation. Several research groups have failed to observe an inhibition of adenylyl cyclase activity in different tissues and cells (Garbarg et al., 1989; Schlicker et al., 1991; Cherifi et al., 1992), which might indicate that H_3-receptors preferentially couple to G_o proteins. There is one interesting report of a negative coupling to phospholipase C in the HGT-1 gastric tumor cell line (Cherifi et al., 1992), but this observation needs confirmation by other research.

V. Other Responses to Histamine

A. Potentiation of Responses to N-Methyl-D-Aspartate

Studies in hippocampal cell cultures, acutely dissociated neurons, and *Xenopus* oocytes expressing the re-

combinant N-methyl-D-aspartate (NMDA) receptor subunits NR2B and NR1 have shown that histamine is able to enhance NMDA-activated currents, independently of the known histamine receptors, via a mechanism that probably involves the polyamine-binding site on the NMDA-receptor complex (Bekkers, 1993; Vorobjev et al., 1993; Williams, 1994; Saysbasili et al., 1995). Histamine and the polyamines spermine and spermidine have also been shown to enhance glutamate toxicity in human NT2-N neurons (Munir et al., 1996). Interestingly, attempts to demonstrate a similar effect of histamine on NMDA-induced currents in rat hippocampal slices, or outside-out patches pulled from the somas of these cells, were without success (Bekkers et al., 1996). However, two studies using conventional and whole cell recording of neurons in the CA1 region of slices of rat hippocampus concluded that the modulation of NMDA-mediated synaptic currents was dependent upon pH (Saysbasili et al., 1995; Janovsky et al., 1995). Thus, at low pH (7.2), histamine enhanced synaptic currents, whereas at pH 7.6 it reduced them. Interestingly, at physiological pH (7.4), no significant action of histamine was seen (Saysbasili et al., 1995).

B. A Role as an Intracellular Messenger?

Although most actions of histamine can be attributed to an extracellular action, there are reports that histamine may have intracellular actions. The activity of the enzyme, histidine decarboxylase, which catalyzes the formation of histamine from histidine, has been observed to be high in several tissues undergoing rapid growth or repair (Ishikawa et al., 1970; Kahlson and Rosengren, 1971; Watanabe et al., 1981; Bartholeyns and Bouclier, 1984; Bartholeyns and Fozard, 1985). These observations have led to the proposal that newly synthesized (nascent) histamine may have a role in cellular proliferation, perhaps via an intracellular site. Some evidence has been accumulated that intracellular histamine levels (or the activity of histidine decarboxylase) can be regulated by tumor-promoting phorbol esters (Saxena et al., 1989). Furthermore, Brandes and colleagues (Saxena et al., 1989; Brandes et al., 1990, 1992) have suggested that N, N-diethyl-2-[4-(phenylmethyl)-phenoxy]ethanamine (DPPE) might be an inhibitor of a specific intracellular histamine receptor (H_{IC}). However, at the present time, the evidence in favor of an intracellular histamine receptor has not been generally accepted, and alternative possibilities need to be explored. For example, the direct effects of histamine, or its analogues, on polyamine sites (Vorobjev et al., 1993; Bekkers, 1993) and heterotrimeric G-proteins (Hagelüken et al., 1995; Seifert et al., 1994) could explain many of the observations to date.

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the underlying membrane mechanism was not studied, it was suggested that HA influenced a voltage-gated conductance via Ca^{2+} translocation.

Other excitatory responses mediated by H_1 receptors occur on cultured tuberal hypothalamic neurons (244, 246) or on hippocampal slices in which slow depolarizations without conductance changes are observed; in addition, on the latter preparation, HA augments excitatory postsynaptic potentials (EPSP) in the CA1 and CA3 areas, presumably via a presynaptic effect (675, 676). In the suprachiasmatic nucleus of slices of rat hypothalamus, local activation of H_1 receptors (similar to that of α_1-adrenergic receptors) inhibits the negative wave elicited by stimulation of the optic nerve (439). On astrocytes of cultured rat brain stem and spinal cord, H_1-receptor stimulation mainly causes depolarizations (325). Excitatory actions of HA after iontophoresis to various brain areas were reported (112, 272, 281, 285, 608), but their possible mediation by H_1 receptors was not shown.

B. Histamine H_2 Receptors

1. Molecular properties

Until very recently a selective labeling of the H_2 receptor was not achieved because of the limited affinity of available ligands. For instance, the saturable binding of [^{3}H]cimetidine to cerebral membranes was assumed to occur at H_2 receptors (102, 164, 377, 384, 718), but neither the affinity of the tritiated ligand nor the potency of competing agents corresponds to that expected from biological responses mediated by H_2 receptors (613, 693, 800). Tritiated ranitidine (92), [^{3}H]impromidine (665), or [^{3}H]ICIA 5165 (500) also proved to be unsuitable.

The utilization of [^{3}H]HA itself as a ligand has led to complex and somewhat perplexing results. Tritiated histamine binds with high affinity, i.e., with a K_D of 2-10 nM depending on the conditions, to cerebral membranes (43, 149, 542, 663, 704-706, 814) and to gastric mucosal cells (50, 51). In early studies with cerebral membranes, the binding appeared to occur to receptors, as shown by *1*) its sensitivity to guanylnucleotides, *2*) its neuronal localization suggested by the effects of kainate lesions, *3*) its marked regional heterogeneity, and *4*) its localization to synaptosomal membranes (43, 542). However, whereas histaminic drugs were active as competing agents, their potencies did not correspond to those at either H_1 or H_2 receptors (or at H_3 receptors that were not yet identified at this time), and it was suggested that the binding occurred at one of the HA receptors in the desensitized state. More recently in an extensive study in which binding parameters were widely varied, several components were distinguished, among which was one characterized by potencies of agonists (but not antagonists) corresponding to those at H_2 receptors. It was proposed that the binding occurred to an allosteric site of the H_2 receptor, but this remains to be established in functional studies (704-706). Whatever the site to which [^{3}H]HA binds, the ligand cannot be used to reliably label the H_2 receptor in biochemical or autoradiographic studies.

Although in several laboratories the antagonist [^{3}H]tiotidine had not been found suitable for labeling the H_2 receptor (49, 52, 442, 666), with the use of a more purified batch of the tritiated ligand, a fraction (~30-40%) of its binding to membranes of guinea pig brain (225, 364, 510, 612, 694, 711) or lung (217) apparently occurred to H_2 receptors, as judged from the potencies of antagonists. In contrast, the K_i values of HA and agonists were 10- to 100-fold higher than their half-maximal inhibitory concentration (IC_{50}) for biological responses mediated by H_2 receptors (including adenylate cyclase activation), implying that the latter occurs with a very large receptor reserve (225). Although significant binding could be detected in three brain areas (cerebral cortex, striatum, hippocampus), this was not the case for other areas or peripheral tissues known to contain H_2 receptors, presumably due to a high nonspecific binding.

More recently, [^{125}I]iodoaminopotentidine, a ^{125}I antagonist, was designed as a highly potent (K_D = 0.3 nM), sensitive, and selective ligand for H_2 receptors (624a, 754a). With this probe the nonspecific binding represented <20-30%, which allowed it to be used not only in biochemical but also in autoradiographic studies (456a, 624a). The corresponding azido derivative was designed as a photoaffinity probe for the H_2 receptor and was shown to bind irreversibly to 58- and 32-kDa peptides isolated by electrophoresis. The H_2-receptor ligands prevented the labeling of these peptides, which were therefore identified as containing the HA recognition domain of the H_2 receptor (624a).

2. Adenylate cyclase activation

Several years before the definition of H_2 receptors and the design of selective antagonists, HA was shown to stimulate adenylate cyclase in broken cell preparations from guinea pig heart, and this effect was shown to be blocked by antihistamines in concentrations well above those required for interaction with H_1 receptors (398, 432, 465). As summarized (153, 487), in brain, most early studies were performed with slices in which the responses are much larger than in membranes, e.g., in rabbits (218, 371), guinea pigs (686), rats (653) or humans (409, 687), but they are more complex since, with the exception of the chick (481, 482), they do not involve a single receptor (see sect. v*A4*).

However, despite its limited stimulation (2- to 3-fold) on cell-free preparations from guinea pig hippocampus, a HA-sensitive adenylate cyclase could be characterized biochemically and pharmacologically as being selectively linked to the H_2 receptor (257, 298, 333, 379). Although rat brain adenylate cyclase is much less sensitive to HA than is guinea pig, some stimulation was reported (257, 298, 548, 580). In several peripheral

sues, e.g., heart or stomach, where biological re-
nses mediated by H_2 receptors occur, a HA-sensitive
nylate cyclase, also selectively linked to the H_2 re-
tor, was characterized (for review see Ref. 363). Fur-
rmore, a number of H_2 receptor-mediated responses
mimicked by cAMP analogues and potentiated by
sphodiesterase inhibitors, suggesting that cAMP is
universal second messenger of HA at H_2 receptors.

The enzyme is activated by HA, which, in rather
concentration (EC_{50} = 10 μM), increases its maximal
ction velocity without altering the K_m for its sub-
ate Mg-ATP. The nucleotide guanosine triphosphate
mulates the enzyme and markedly potentiates the
-induced stimulation, indicating that the ternary
del (617) comprised of the H_2 receptor (the HA recog-
ion subunit), a GTP regulatory protein (G_s) (the
nsducer), and adenylate cyclase (the catalytic unit)
olies to this system. Free Mg^{2+} stimulates the cata-
ic activity presumably by interacting with an allos-
ic site of the enzyme, the affinity of which might be
reased on HA stimulation (379).

The adenylate cyclase response in membranes of
nea pig brain is triggered by H_2-receptor agonists
l competitively inhibited by typical H_2-receptor an-
onists at concentrations closely consistent with
se required at H_2 receptors mediating various biologi-
responses (257, 298). However, an intriguing obser-
ion is that a series of psychotropic compounds,
nely several neuroleptics and a number of struc-
ally diverse antidepressants, behave as extremely
ent and competitive inhibitors of the response to HA
cerebral membranes (257, 259, 378). This has led to
suggestion that blockade of the H_2 receptor repre-
ts the molecular basis of clinical antidepressant ac-
ity (378). However, on intact cell preparations, i.e.,
pocampal slices (156) or dissociated brain tissues
4), stimulated by selective H_2-receptor agonists,
se compounds are relatively weak antagonists. The
sons for these intriguing differences are not known,
l stimulation of adenylate cyclase in membranes
uld not be regarded as a reliable system to assess the
ivity of compounds at H_2 receptors. In addition, the
a that antidepressants derive their clinical efficacy
m blockade of cerebral H_2 receptors seems unlikely,
ticularly since such a blockade could not be observed
er chronic treatments (514).

In several systems increased intracellular cAMP
tent leads to final biological responses via enhanced
sphorylation of intracellular proteins. However, al-
ugh stimulation of H_2 receptors in gastric parietal
ls was shown to selectively activate a cytosolic type I
MP-dependent protein kinase (124) and to stimulate
phosphorylation of several cytosolic proteins (125,
), the corresponding effects on cerebral H_2 receptors
re not reported. These intracellular steps mediated
cAMP might be regulatory steps, because electro-
siologically hypersensitive responses to HA elicited
denervation (286) or chronic antidepressant treat-
nts (514) are not accompanied by changes in respon-
ness of the cAMP system to HA.

3. *Phospholipid methylation and other biochemical responses*

Hirata and Axelrod (316) suggested that methylation of membrane phospholipids plays a role in signal transduction across membranes. Stimulation of H_2 receptors in mast cell membranes (754) and synaptic membranes of rat brain (536, 537) rapidly increases incorporation of [^{3}H]methyl groups from *S*-[^{3}H]adenosylmethionine into phospholipids. On the latter preparation, the EC_{50} of HA was 5 μM, and concentration-response curves obtained with several agonists and antagonists rather clearly identified the H_2 receptor as mediating the response. Although controversial (836a), release of endogenous norepinephrine might be modulated by H_2 receptors (79a). The absence of correlation between the density of binding sites and adenylate cyclase activity (624a) might reflect the existence of H_2-receptor subtypes, possibly linked to these various responses.

4. *Localization*

Until recently the information on H_2-receptor localization in the brain was essentially derived from studies of the cAMP response they mediate. The fact that this response occurs in neurons is suggested by several observations: *1*) the HA-sensitive adenylate cyclase is enriched in synaptic membranes isolated from guinea pig cerebral cortex (379); *2*) the cAMP response occurs on various fractions enriched in neuronal elements, e.g., the vesicular sacs called synaptoneurosomes (229, 230, 322, 323, 548, 586, 587); and *3*) kainate lesions in the hippocampus nearly abolish the response in slices (234), whereas interruption of HA afferents does not affect it (286). The prenatal development pattern of the cAMP response is also consistent with a neuronal localization of H_2 receptors (689).

The cAMP response involving H_2 receptors is not limited to neurons, since it can be also evidenced in human astrocytoma cells (129), epithelial cells of choroid plexus (148), and cerebral microvessel preparations (341, 367, 382, 546, 547). However, a clear cAMP response can only be measured in a few brain areas of a few animal species, and the amplitude of the response (153, 670) does not parallel the relative densities of histaminergic afferents, which suggests that mismatches exist for not only H_1 but also H_2 receptors.

In contrast with binding data obtained with [^{3}H]tiotidine (612, 694), autoradiographic mapping of the H_2 receptor in guinea pig or rat brains using ^{125}I-aminopotentidine has shown it to be distributed widely and in a highly heterogeneous fashion, e.g., with clearcut differences among laminae of cortical areas, suggesting its major association with neurons (624a).

The H_2 receptors are present in most areas of the cerebral cortex, with the highest density in the superficial layers, the piriform and occipital cortex, which contain low H_1-receptor density. The caudate putamen (which contains low H_1-receptor density), nucleus accumbens, and olfactory tubercles are among the richest

brain areas. In the hippocampal formation, H_2 receptors display a laminated pattern with dense labeling in the lacunosum moleculare, radiatum, and oriens layers. In the thalamus the low density of H_2 receptors contrasts with the high density of H_1 receptors. The H_2 receptors are not abundant in the hypothalamus (where HA axons are the densest) nor in most other brain areas except the superficial gray layer of colliculus superior and, to a lesser extent, the substantia nigra. These distributions suggest that H_1 and H_2 receptors may mediate together the actions of HA in certain brain areas, e.g., cerebral cortex or hippocampus, whereas in other areas, e.g., thalamus or caudate putamen, a single subtype might be primarily involve. The H_2 receptors are hardly detectable in pituitary.

5. Electrophysiological responses

In early studies in which HA was applied by microintophoresis on many neurons all over the CNS of anesthetized animals, the response most frequently found was an inhibition of firing, with both fast onset and fast recovery (for review see Refs. 276, 277). This pattern was found on neurons from the cerebral cortex (279, 285, 563, 564, 639), the brain stem reticular formation (89, 278), the dorsal raphe nucleus (427, 428), the cerebellum (458, 690), the vestibular nucleus (391, 641), the thalamus and hippocampus (273, 285), the ventromedial nucleus of the hypothalamus (608), and the preoptic area (112). These depressive actions may be mediated by H_2 receptors, as suggested in some of these studies by the blockade exerted by burimamide, metiamide, or cimetidine. However, this conclusion is only a tentative one, since *1*) the iontophoretic application does not allow one to determine the apparent affinities of antagonists, *2*) metiamide and even more burimamide are now known to display very significant H_3-receptor antagonist potency, and *3*) the highly selective H_2-receptor agonists dimaprit and impromidine were generally not tested.

Iontophoretically applied metiamide also antagonizes, at least partly, the depressant responses of cerebral cortical neurons elicited by stimulation of the medial forebrain bundle, of hippocampal cortical neurons elicited by stimulation of the fornix (284, 285, 640), or of nucleus accumbens neurons evoked by stimulation of the afferent fimbria (126). These various data suggest that H_2 receptors mediate the inhibitions elicited by endogenous HA, a view supported by the clear hypersensitive inhibitory response that develops in the guinea pig sensorimotor cortex after lesions of the ascending HA pathway (286).

On unidentified cells of explants of tuberal hypothalamus, iontophoretic application of HA also elicits depressant effects with a rapid time course, which are clearly mediated by H_2 receptors; furthermore the persistence of the response in a Ca^{2+}-free medium and its potentiation by phosphodiesterase inhibitors suggest that it is postsynaptically mediated by an increase in cAMP (245, 246). Histamine hyperpolarizes human spinal neurons in culture (588). A similar effect is mediated by H_2 receptors on astrocytes of cultured rat brain stem and spinal cord (326) and on suprachiasmatic neurons of rat hypothalamic slices (439).

In slices of rat hippocampus, local pressure or iontophoretic application of HA or impromidine to pyramidal or granule cells hyperpolarizes these cells via a presumably postsynaptic effect (273). Perhaps more important effects, apparently mediated by H_2 receptors, were evidenced on the same preparation by Haas and colleagues (274, 276, 280, 282, 283) when HA or impromidine was added to the perfusion fluid and when CA1 pyramidal neurons were recorded intracellularly. Histamine caused a slight depolarization without change in resting membrane conductance, an abbreviation of long afterhyperpolarizations, and a loss of accommodation of action potential firing to excitatory inputs. The two latter actions result in a strong potentiation by HA of various excitatory signals, e.g., depolarization induced by excitatory amino acids and synaptically evoked spikes, by inhibition of their self-restriction processes. These effects were attributed to a decrease of a Ca^{2+}-activated K^+ conductance (283), a mechanism confirmed by voltage-clamp studies (556) and that may also underly the action of HA at medullary (90, 366) or myenteric neurons (490). A similar mechanism might be responsible for the H_2 receptor-mediated enhancement of excitability of CA3 pyramidal neurons treated with penicillin (523) or after stimulation of mossy fibers (729). These indirect excitatory effects might depend on intracellular cAMP inasmuch as they are potentiated by a phosphodiesterase inhibitor and mimicked by intracellular application of the nucleotide (275) or activation of the cell's adenylate cyclase by forskolin (499).

Abbreviation of afterhyperpolarization in the same cells triggered by stimulation of β_1-adrenergic receptors also occurs via a similar mechanism (499). A similar effect elicited by stimulation of muscarinic receptors (59) might result from intracellular protein kinase C activation (39). Therefore it appears that inhibition of a Ca^{2+}-dependent K^+ conductance might represent the final common path for the actions of various neurotransmitters released from several highly divergent neuronal pathways. Hence, as underlined (276), these essentially modulatory actions of HA, consisting of a weak direct effect but a strong potentiation of excitatory signals, suggest that the function of the amine in brain is not to transmit discrete information but to regulate in a coordinate manner the excitability of large cerebral areas (655, 660).

C. Histamine H_3 Receptors

1. Molecular properties

The highly potent agonist *R*-α-[^{3}H]methylhistamine (MeHA) constitutes a suitable probe for the selective labeling of the H_3 receptor (23). In the absence o divalent cations, *R*-α-[^{3}H]MeHA binds in a saturabl

anner to an apparently homogeneous population of es in membranes of rat cerebral cortex. The binding is versible, as indicated by the similar dissociation con-ants (K_D = 0.3 nM) derived from either association-ssociation kinetics or saturation kinetics at equilib-um (K_D = 0.4 nM). These binding sites are pharmaco-gically identified as H_3 receptors by competition udies performed with various H_1, H_2, or H_3 histamin-gic drugs (28a). The relative potencies of various ago-sts and affinity constants of various antagonists are ghly correlated with the corresponding values ob-ined at functional H_3 autoreceptors regulating HA re-ase or synthesis. For instance, the *R*-isomer of α-eHA is ~10 times as potent as HA itself, and *S*-α-eHA is ~100 times less potent than *R*-α-MeHA. This nfirms that enantiomers corresponding to *S*-config-ated L-histidine are highly preferred at H_3 receptors, ereas enantiomers corresponding to D-histidine are ore potent at H_2 receptors, with no difference being served at H_1 receptors (23, 29). The potent and specific H_1-receptor antagonist thioperamide (23), the H_1-recep-r agonist betahistine (24), the H_2-receptor agonist im-omidine (25), and the psychoactive drug phencyclidine l) inhibit *R*-α-[^{3}H]MeHA binding with the same affin-y as that displayed when tested as antagonists at func-nal H_3 autoreceptors.

Two affinity states of the H_3 receptor are observed en the binding of *R*-α-[^{3}H]MeHA is studied in the esence of Ca^{2+} in physiological concentrations, an ef-ct that is not reproduced by Mg^{2+} and apparently ises from the conversion of a major fraction of H_3 ceptors to a lower affinity state (K_D = 16 nM). The nding of the tritiated ligand to this low-affinity com-nent is entirely and specifically inhibited in the pres-ce of guanylnucleotides, indicating that the H_3 recep-r is coupled to its (so far unknown) effector system via G protein. However, at numerous other receptor types, anylnucleotides affect binding to the high- and not e low-affinity component (28a). The low-affinity com-nent may correspond to the functionally active recep-r, since the K_D value of *R*-α-MeHA at this component close to its EC_{50} values at H_3 autoreceptors regulating A release and synthesis. However, the functional in-lvement of the high-affinity population of sites can-t be entirely excluded, since it also appears to be (al-ough very partially) GTP regulated. The 10 times gher affinity of agonists to this high-affinity compo-nt compared with their potency in functional studies ggests that these sites may represent the H_3 receptor a modified (desensitized?) state.

Distribution in central nervous system

The mean density of cerebral *R*-α-[^{3}H]MeHA bind-g sites determined in physiological conditions in rats rather low, i.e., ~30 fmol/mg membrane protein. It is ightly higher in the guinea pig brain, suggesting that e number of H_3 receptors, similar to that of H_1 recep-rs, varies among species. The H_3 receptors mediate the autoinhibition of HA release in human brain with a pharmacology apparently similar to that of correspond-ing receptors in rodents (22).

The distribution of H_3 receptors in rat brain, estab-lished from either membrane binding studies or autora-diographic studies, is highly heterogeneous (23; H. Pol-lard, J. Moreau, J. M. Arrang, and J. C. Schwartz, un-published observations; Fig. 2).

In cerebral cortex where they are rather dense, H_3 receptors are found in all areas and layers with, how-ever, a higher abundance in rostral areas and in laminae IV and V. In the hippocampal formation, they are moder-ately to highly abundant, with their density being the highest in the dentate gyrus, moderate in subiculum, and very low in the fimbria. In the amygdaloid complex high densities are found in central, lateral, and baso-lateral nuclei as well as in the bed nucleus of the stria terminalis, which contains a dense histaminergic inner-vation (60).

In the basal forebrain, numerous H_3 receptors are present in anterior olfactory nuclei, nucleus accumbens, and olfactory tubercles, as well as in striatum, particu-larly in its dorsomedial part; they are less numerous in the globus pallidus and even less in the septum. In the thalamus, H_3 receptors are mainly detected in various midline, intralaminar, and lateral nuclei. In the hypo-thalamus, their moderate density contrasts with the high density of HA axons, but they are detectable at the level of the tuberomammillary nucleus where they may reside on perikarya or dendrites (28). In the mesencepha-lon, they are numerous in the substantia nigra, particu-larly in its pars reticulata, the ventral tegmental area, and superior colliculi. In cerebellum, low densities are present in all layers. In the brain stem, they are mainly present in pontine nuclei, around the fourth ventricle, in locus coeruleus, and in the dorsal tegmental nucleus. In the spinal cord, a low density is present mainly in exter-nal layers of the dorsal horn.

This distribution of H_3 receptors, not strictly paral-lel to that of histaminergic axons, suggests that they are not restricted to the latter. This is confirmed by the identification of H_3 receptors on serotoninergic and nor-adrenergic nerve terminals in the cerebral cortex (652, 652a) and a cerebral vessel (197). Their decrease elicited in striatum by local administration of the neurotoxin kainate is consistent with a major neuronal localization (Pollard et al., unpublished observations).

In the vegetative nervous system, H_3 receptors with a presynaptic localization have been evidenced at the level of the mesenteric artery where they mediate inhibi-tion of excitatory junction potentials generated by stim-ulation of sympathetic perivascular nerves (354). Also, their stimulation inhibits transmission at nicotinic syn-apses of enteric ganglia (737), an effect that may be re-sponsible for the inhibition of electrically evoked con-tractions of the ileum (762). Presynaptic H_3 receptors on vagal nerve endings might also be responsible for the inhibition of cholinergic transmission in airways (348a, 349) and of gastric acid secretion (301, 302). Presynaptic H_3 receptors might also mediate inhibition of neuropep-

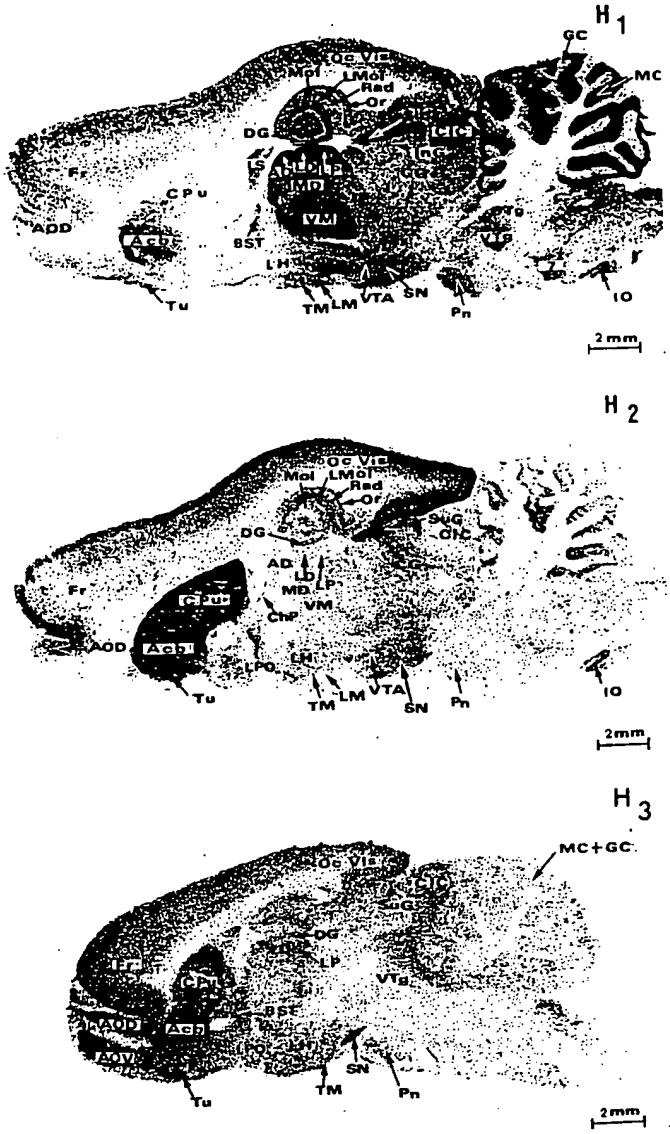

FIG. 2. Autoradiographic localization of histamine receptors on midsagittal sections of brain. H_1 and H_2 receptors were visualized on sections of guinea pig brain using [^{125}I]iodobolpyramine and [^{125}I]iodoaminopotentidine, respectively. (Courtesy of M. L. Bouthenet.) H_3 receptors were visualized on section of rat brain using *R*-α-[^{3}H]methylhistamine. Abbreviations: ACb, nucleus accumbens; AD, anterodorsal thalamic nucleus; AOD, anterior olfactory nucleus, dorsal part; AOV, anterior olfactory nucleus, ventral part; BST, bed nucleus of stria terminalis; CIC, central nucleus of inferior colliculus; CG, central gray; ChP, choroid plexuses; CPu, caudate putamen; DG, dentate gyrus; DTg, dorsal tegmental nucleus; Fr, frontal cortex; GC, granular layer of cerebellum; InG, intermediate gray layer of superior colliculus; IO, inferior olive; LD, laterodorsal thalamic nucleus; LH, lateral hypothalamic area; LM, lateral mammillary nucleus; LMol, lacunosum molecular layer of hippocampus; LP, lateral posterior thalamic nucleus; LPO, lateral preoptic area; LS, lateral septum; MC, molecular layer of cerebellum; MD, mediodorsal thalamic nucleus; Mol, molecular layer of hippocampus; Oc Vis, occipital visual cortex; Or, oriens layer of hippocampus; Pn, pontine nuclei; Rad, stratum radiatum of hippocampus; SN, substantia nigra; SuG, superficial gray layer of superior colliculus; TM, tuberomammillary nucleus; Tu, olfactory tubercle; VM, ventromedial thalamic nucleus; VTA, ventral tegmental area; VTg, ventral tegmental nucleus; 7, 7th cranial nerve.

tide release from airway sensory nerves (348b, 348c). Hence H_3 receptors are not restricted to the brain.

3. *Actions mediated by H_3 receptors*

The best established actions mediated by H_3 receptors are the presynaptic inhibitions of release of several neurotransmitters, i.e., HA, 5-HT, and norepinephrine in the brain and possibly acetylcholine, norepinephrine, and neuropeptides in the peripheral nervous system. As in the case of other presynaptic receptors, these actions may arise from a restriction of Ca^{2+} influx related to increases in hyperpolarizing conductances, possibly mediated by changes in channel phosphorylations resulting from inhibition of adenylate cyclase (511).

Stimulation of H_3 receptors decreases wakefulness in cats (369, 437a) and spontaneous motor activity in rats (93). It induces vasodilation of the rabbit isolated middle cerebral artery (197).

Nonneuronal (mast cell?) H_3 receptors mediate the inhibition of HA synthesis in various peripheral tissues (23).

VI. ROLE OF HISTAMINERGIC NEURONS IN NEUROENDOCRINE CONTROLS

Both the morphology of HA perikarya, recalling that of magnocellular secretory neurons in the anterior hypothalamus, and the high density of their projections to hypothalamic areas involved in the regulation of hormonal secretions are consistent with a participation of endogenous HA in neuroendocrine controls. In agreement with this hypothesis, exogenous HA elicits a variety of hormonal responses in vitro or in vivo, but the role of endogenous HA has been difficult to demonstrate (for reviews see Refs. 178, 179, 770, 810).

Systemic administration of HA elicits marked biological responses, such as hypotension or adrenal hormone secretion, which in turn may indirectly affect neuroendocrine controls. Because such changes may not involve histaminergic systems in the brain, they are generally not considered here.

A. *Posterior Pituitary Hormone Secretion*

Both the supraoptic and paraventricular nuclei of the hypothalamus, which synthesize and control the release of vasopressin and oxytocin, contain high levels of HA (98) and HDC activity (567) and high densities of HA axons (551). The neurohypophysis itself contains, mainly in its peripheral part, HDC- and HA-immunoreactive fibers, presumably arising from the TM and passing through the internal layers of the median eminence and the infundibular stalk (351). The H_1 receptors are extremely abundant in the supraoptic nucleus and present in the paraventricular nucleus (86, 543).

In several animal species, intracerebroventricular administration of HA elicits antidiuretic responses (61,

1, 317, 431, 772, 773), which seem to result from ased vasopressin secretion (69, 71, 171, 775, 776). effects of various agonists and antagonists indicate this action is selectively mediated by H_1 receptors 317, 776).

ontophoretic application of HA to the supraoptic us increases the firing of the neurosecretory cells via H_1 receptors, but the possibility of a distal effect raised (285), and no effects were observed by others 331). In perfused rat hypothalamoneurohypophy- explants, HA excited and facilitated the burst activ- antidromically stimulated supraoptic neurons; the ts occurred via H_1 receptors and appeared to de- on the electrical activity of the neuron, suggesting HA affected a voltage-dependent conductance (19). Although the effects of exogenous HA seem well olished, this is not the case for a possible participa- of the endogenous amine in the control of vasopres- elease. In Brattleboro rats genetically lacking vaso- sin, the HA levels are elevated in several hypotha- c nuclei, including the supraoptic nucleus, and eased in the neurohypophysis, but the functional ficance of these findings is obscure (139). Pituitary pressin depletion induced by repeated administra- of hypertonic saline was accompanied by a signifi- decrease in HA levels in neurohypophysis (785), esting that the control of vasopressin secretion by night occur at the level of both perikarya and termi- of the neurosecretory neurons. Increased hypotha- c HA, induced by chronic His loads in rats, de- sed vasopressin levels in the anterior hypothalamus lid not modify the hormone level in plasma; chronic bition of HA synthesis by α-FMH impaired the va- essin response to adrenalectomy but did not modify ificantly vasopressin secretion in controls (107). ce HA neurons might control vasopressin secretion r certain circumstances, but since changes in basal tion do not occur after HA depletion and were r reported after administration of H_1-receptor an- nists alone, endogenous HA may not exert a tonic ence on neurosecretion under basal conditions.

The secretion of oxytocin is also enhanced after in- erebroventricular administration of HA, but high s are necessary (171).

rolactin Secretion

Secretion of this hormone by the lactotrophs of the ohypophysis is mainly controlled, in a tonic inhibi- fashion, by dopamine released from tuberoinfun- lar neurons into the median eminence capillaries of portal system. There are various indications that might be involved in the control of prolactin secre-

Intracerebroventricular administration of HA in or ovariectomized estradiol-primed rats increases lasma levels of prolactin (18, 176, 180, 184, 435, 614). avenous HA elicits similar responses in steroid- ed rats (614), in rhesus monkeys (193), and in human males (400, 406, 578). This effect does not appear to be exerted at the level of the anterior pituitary, because it is not observed after local injections or applications of HA (435, 614). The anterior pituitary does not contain irHA axons (351), and H_1 or H_2 receptors appear scarce (M. L. Bouthenet, unpublished observations).

Histamine may act on central structures in the vicinity of the third ventricle, because the threshold doses of the amine are lower when given in the ventricle than in the lateral ventricle or systemically (178) and because prolactin secretion is also elicited by HA administered into the anterior hypothalamic area or the arcuate nucleus region (13, 15, 435). Histamine infused centrally decreases the secretion of dopamine into the pituitary portal blood of female (248) and male rats (404, 405). Dopamine receptor blockade by pimozide did not prevent the rise in plasma prolactin elicited by intracerebroventricular administration of HA (404). Hence the prolactin-stimulating effect of HA might occur through an inhibition of the tuberoinfundibular dopaminergic neurons. Both the arcuate nucleus and the median eminence contain irHA and irHDC axons (351) and H_1 receptors in moderate density (86, 543, 681). Receptor antagonists for 5-HT partially prevent the action of HA, suggesting a participation of the endogenous indolamine (405). The rat median eminence, which is located outside the blood-brain barrier, also contains mast cells depletable by compound 48/80 (567), and injection of this compound systemically, but not intraventricularly, increases prolactin secretion (182).

The receptor subtypes mediating the effect of HA have been subject to some controversy (for detailed discussion see Refs. 177, 178). The rise in prolactin secretion elicited by HA in rats is blocked by various H_2-receptor antagonists injected intracerebroventricularly (183, 185, 186, 400, 402, 403) and mimicked by dimaprit, a selective H_2-receptor agonist (186, 401). However, dimaprit was also shown to reduce plasma prolactin (186, 209). Intra-arterial administration of cimetidine or ranitidine, two H_2 antagonists that do not cross easily the blood-brain barrier, partially blocked the effect of intracerebroventricularly administered HA (403).

A possible participation of H_1 receptors in the HA-induced secretion of prolactin is less clear than that of H_2 receptors. The H_1-receptor antagonists, such as mepyramine, that easily cross the blood-brain barrier (591) partially block the effect of centrally administered HA when they are administered intracerebroventricularly in relatively high dosages (180, 403) but do not block it when administered intravenously (405). Hence a nonspecific effect of the H_1-receptor antagonists administered centrally, e.g., via membrane stabilization, seems likely. In contrast, systemically administered H_1 antagonists appear to block the prolactin secretion induced by intravenous HA, but the latter effect might be indirect and not mediated by cerebral receptors (406, 443, 614).

A possible role of endogenous HA in the control of prolactin release under various circumstances was investigated using receptor antagonists or a synthesis in-

hibitor as pharmacological tools. The H_1-receptor antagonists administered systemically do not modify basal prolactin secretion in male rats (403) or in humans (406). In contrast, the H_2-receptor antagonist cimetidine, given intravenously, promotes prolactin secretion in humans (104, 114, 406) or in rats (492), and chronic treatments with the drug produces gynecomastia and galactorrhea accompanied by hyperprolactinemia in a few patients (48, 163). These observations would not be consistent with a role of HA in promoting prolactin secretion via H_2-receptor stimulation, but other observations suggest that the effect of cimetidine is not related to H_2-receptor blockade. Hence the effect of cimetidine was not modified by the H_2 agonist impromidine (681); in addition, ranitidine was less effective than cimetidine, although it is a more potent H_2 antagonist, and oxmetidine, another H_2 antagonist, seems completely ineffective (407, 492, 683). Finally, a compound resembling cimetidine in chemical structure but devoid of H_2-antagonist activity stimulated prolactin secretion (402). Because most H_2 antagonists do not easily cross the blood-brain barrier, it will be of interest to assess the action of the brain-penetrating antagonist zolantidine (108).

Depletion of hypothalamic HA by α-FMH (235) is accompanied by a decrease in basal plasma prolactin levels (495).

The rises in plasma prolactin elicited in male rats by restraint stress or acute exposure to ether vapors is prevented by several H_2 antagonists administered intracerebroventricularly as well as by α-FMH (12, 403, 495, 678). The H_1 antagonists administered systemically partially decrease the prolactin responses (12, 403). In contrast, the prolactin response triggered by suckling of lactating rats or by estrogens is blunted by H_1 antagonists but is not affected by H_2 antagonists (13, 18).

Taken together and despite several inconsistencies, these various studies suggest that cerebral histaminergic neurons participate in the control of prolactin secretion with a major involvement of H_2 receptors, but the participation of H_3 receptors remains to be assessed.

C. *Thyrotropin Secretion*

Thyrotropin (TSH) secretion by the adenohypophysis is controlled in a stimulatory fashion by thyrotropin-releasing hormone (TRH), a tripeptide released by neurons originating from the paraventricular and periventricular nuclei of the hypothalamus and ending in the external zone of the median eminence in apposition with the capillaries of the portal system. On the contrary, somatostatin, released at the same level from neurons originating from the periventricular area, inhibits TSH secretion.

Histamine might be involved in control of TSH secretion (for review see Ref. 765).

Intracerebroventricular administration of HA does not change basal TSH secretion (319, 443, 768) but decreases TSH secretion induced by either exogenous TRH (768) or by a cold stress (764, 766, 768, 769). Furthermore, His, in doses large enough to increase cerebral HA (668), inhibits the cold-stimulated (but not the TRH induced) TSH response, suggesting a role for endogenous HA (769). From the effects of focal injections, HA appears to act at the level of the periventricular hypothalamus (768) and not of the pituitary, since the amine did not modify basal or TRH-stimulated release of TSH from superfused rat anterior pituitary cells (767) and since dimaprit did not modify TSH release from anterior pituitary halves in vitro (167). The inhibition of the cold response by HA cannot be ascribed to an impaired release of TRH, since the amine actually liberates TRH from hypothalamic slices (123, 368) or synaptosomes (62), with both effects being apparently mediated by H_2 receptors. Theoretically the action of HA might result from somatostatin release, but this effect is not substantiated by experimental evidence. It is not clear whether the effect of HA on the cold response is receptor mediated since it was mimicked by impromidine or 2-pyridylethylamine but not antagonized by either mepyramine or cimetidine (768, 769). In contrast, systemic administration of the selective H_2 agonist dimaprit reduced serum TSH basal level in a cimetidine-reversible manner (167).

Furthermore, mepyramine and cimetidine administered alone do not modify the cold-induced secretion of TSH (768), suggesting that endogenous HA is not involved.

D. *Growth Hormone Secretion*

Growth hormone (GH) secretion is controlled in opposite fashions by GH-releasing factor (GRF) and somatostatin.

In unanesthetized male rats, intracerebroventricular administration of HA suppresses the spontaneous pulses of GH secretion, and this effect is partially mimicked by an inhibitor of HMT (491). This effect was not observed in rats in which most of the somatostatinergic innervation of the median eminence was eliminated (372), which may suggest that HA triggers somatostatin release. However, a somatostatin antiserum did not prevent the suppression of GH pulses by HA (493).

In anesthetized dogs, intracerebroventricular HA reduces GH secretion only slightly (625). In rats, HA also diminishes the GH secretion elicited by morphine, an action apparently mediated by H_1 receptors (494). Intracerebroventricular administration of GRF in low dosage elicits significant increases in HA concentrations in rat hypothalamus and pituitary, whereas somatostatin elicits opposite changes (106). These observations suggest reciprocal interactions between somatotropinergic and histaminergic systems in brain, but their relevance to the regulation of GH secretion remains to be clarified.

E. *Adrenocorticotropic Hormone Secretion*

For a long time, a role for HA in the control of adrenocorticotropic hormone (ACTH) secretion has

ı postulated from the numerous observations show- that, on systemic administration, it elicits increases lasma ACTH and corticosterone levels mainly via ıulation of H_1 receptors (178, 770, 810). In addition, ous stressful procedures affect HA turnover in n (see sect. XIII.A). However, a participation of cere- histaminergic neurons in the control of ACTH se- ion largely remains to be clarified, since stimulation eripheral H_1 receptors causes hypotension, a strong ıulus for ACTH secretion.

Intracerebroventricular administration of HA in :s low enough to avoid stimulation of peripheral re- ors increases ACTH and/or corticosteroids secre- in both dogs (625) and rats (99, 101, 677) in which it :companied by an increased secretion of β-endorphin ·). In rats, H_1 receptors alone (677) or together with :eceptors seem involved (99, 101), whereas in dogs ethylhistamine, a predominantly H_2-receptor ago- , decreases ACTH secretion (625). The action of HA ; not appear to result from a direct secretory effect either corticotropin hormone-releasing hormone ', 314) or ACTH (630), as indicated by in vitro studies ı hypothalamic and pituitary tissues, respectively. ıce an indirect action through other monoamines :rolling ACTH secretion has been suggested (100, ·. However, iontophoretically applied HA strongly tes cortisol-inhibitable neurons in the mediobasal othalamus (452).

Controversial data have been obtained regarding involvement of endogenous HA in the regulation of ΓH and corticosteroid secretion.

In rats neither H_1 nor H_2 antagonists prevent the ΓH and corticosterone responses elicited by an acute .raint stress (678) and, in mice, neither His loads nor .bition of HA synthesis by brocresine, a nonspecific ırboxylase inhibitor, modifies the corticosterone re- nse to restraint (789). In contrast, histidine loading .es plasma corticosterone levels in rats (462). Specific ibition of HDC by α-FMH decreases plasma cortico- ·one in nonstressed as well as in stressed rats; how- :, because this effect is not accompanied by any sig- :ant change in plasma ACTH, a direct action of the ibitor at the adrenal level was suggested (678).

In contrast, in humans the H_1 antagonist meclas- : prevents the ACTH response to hypoglycemia or :yrapone, a corticosteroid synthesis inhibitor (9). In ition, in rats treated with α-FMH the ACTH re- nse to adrenalectomy is completely abolished (793).

Gonadotropin Secretion

Secretion of luteinizing hormone (LH) and follicle- ıulating hormone (FSH) are under the control of go- otropin-releasing hormone (GnRH), and the secre- ı of both the pituitary and hypothalamic hormones is lulated by gonadal steroids.

Intracerebroventricular administration of HA in ge doses evokes ovulation in anesthetized rabbits l) and LH release in ovariectomized rats primed with ·ogen and progesterone (176, 184, 435) but not in male rats (181). This effect does not result from a direct stimulation at the pituitary level, since, in vitro, HA does not affect LH release from rat pituitary isolated cells (72) or fragments (123, 470). On the other hand, HA elicited GnRH release from perfused rat mediobasal hypothalamus, and GnRH was able to stimulate LH release from pituitaries sequentially perfused in a second perfusion chamber (470). The effect of HA could be mediated by H_1 receptors, since it was mimicked by 2-methylhistamine but not by 4-methylhistamine, two relatively selective agonists at H_1 and H_2 receptors, respectively, and antagonized by mepyramine, with the latter used, however, in high concentration (10 μM). Although not directly assessed, a possible involvement of endogenous HA in the control of LH release is suggested by the observation that castration increases HA level in the hypothalamus of male rats (529) and HDC activity in the hypothalamus of female rabbits (76) and that brain HA levels fluctuate in the hamster during the estrus cycle and on selected days of pregnancy (315).

VII. ROLE OF HISTAMINERGIC NEURONS IN CARDIOVASCULAR REGULATION

In conscious or anesthetized animals, i.e., rats, cats, or goats, intracerebroventricular administration of HA increases blood pressure accompanied by bradycardia in conscious animals or tachycardia in anesthetized animals (162, 214-216, 241, 396, 397, 453, 581, 691, 761, 775, 819). These effects are not attributable to leakage of HA into the general circulation, because the amine has an hypotensive action when administered peripherally.

The central hypertensive action of HA results only partially from an activation of sympathetic activity, a participation of endogenous vasopressin being suggested by the attenuation of the response observed with a vasopressin antagonist (241). Cerebral catecholaminergic pathways may also contribute to this action, which is reduced after intracerebroventricular administration of the neurotoxin 6-hydrodopamine (214). The hypertensive response to HA might involve both H_1 and H_2 receptors, as suggested by the action of various agonists, including the highly selective agonist impromidine, but the effects of antagonists, often used intracerebroventricularly in extremely large doses, has led to contradictory results (214-216, 303, 453, 538, 581). In contrast to the above effects, HA administered into the C_1 area of the rostral ventrolateral medulla causes hypotension and bradycardia selectively via stimulation of H_2 receptors (253). In cats, HA perfused at high concentration enhances the pressor effect of stimulation of the caudal hypothalamus selectively via H_2 receptors (562).

Endogenous HA might have a role in central cardiovascular regulation. The spontaneous release of HA in superfusates of cat posterior hypothalamus seems to be altered according to modifications of blood pressure (560, 561). In two studies (138, 582), spontaneously hypertensive rats displayed increased HA levels in several hypothalamic areas, e.g., suprachiasmatic or arcuate nucleus and median eminence, but not in various brain

stem areas, whereas others failed to detect any difference (118, 740). In these hypertensive rats, the rate of basal HA release in superfusates of the posterior hypothalamus is significantly increased (779). Elevated brain HA levels induced by an inhibitor of HMT were accompanied by increased blood pressure and bradycardia, but blockade of this response by HA antagonists was not reported (396). In fact, the effects of HA antagonists given alone do not clarify the issue of a possible role of endogenous HA. Whereas most H_1 antagonists crossing the blood-brain barrier display little cardiovascular activity (188), H_2 antagonists administered intracerebroventricularly elicit biphasic or even hypertensive effects similar to HA itself (215, 242, 397). This paradoxical effect might be related to blockade of either brain stem H_2 receptors mediating hypotensive actions (253) or H_3 autoreceptors controlling HA release for which several H_2 antagonists display significant affinity (25).

VIII. ROLE OF HISTAMINERGIC NEURONS IN CONTROL OF CEREBRAL CIRCULATION

A possible role of HA in the control of cerebral circulation is suggested by the presence of mast cells (see sect. III*A*) as well as of HA axons in vicinity of cerebral blood vessels (710). In addition, cerebral microvessels isolated by a sieving procedure contain measurable amounts of HA associated with HDC activity at a very low level (362, 381, 382) as well as H_1 receptors detected by [^{3}H]mepyramine binding (557) and H_2 receptors mediating a cAMP response (38, 381, 382).

Histamine has multiple actions on cerebral blood vessels (for review see Refs. 262, 263, 534, 535).

On pial arteries and arterioles, perivascular microinjection of the amine elicits dilation mainly via activation of H_2 receptors, whereas pial veins and venules seem unresponsive (200, 264, 266, 794). The HA-induced dilation of pial arterioles of newborn pigs seems to be due to a production of vasodilator prostanoids (469).

On precontracted isolated preparations of cat (200, 201, 264) or rabbit middle cerebral arteries (680), HA produces vasodilation apparently mediated by H_2 receptors. This response seems to involve the endothelium, and at higher concentrations vasoconstriction mediated by H_1 receptors is observed. On precontracted human cerebral arteries, dilation involves H_1 and, to a lesser extent, H_2 receptors (532). In the rabbit precontracted preparation in which H_1 and H_2 receptors were blocked, HA in low concentrations still elicited vasodilation via activation of H_3 receptors, and this effect is mimicked by the selective H_3 agonist α-MeHA in extremely low concentration (197). In rats, extraluminally administered HA causes dilation of the spontaneous tone of isolated intracerebral arterioles via activation of H_2 receptors (150).

Intracarotid injection of HA in rats increases cerebral blood flow via activation of both H_1 and H_2 receptors but only after transient disruption of the blood-brain barrier by hypertonic urea (261, 265, 267). This may reflect the fact that the cerebral vessels are more responsive to the amine when the latter is delivered to their external layers (534). In the human brain, however, transient vasodilative responses are observed, even when the blood-brain barrier is intact (750).

Histamine infused into rat internal carotid artery increases, via H_2-receptor stimulation, the extravascular space for sucrose or horseradish peroxidase as well as the cerebral water content; electron-microscopic studies showed pericapillary astroglial processes to be swollen, consistent with cerebral edema (84, 196, 267). Blockade of H_2 receptors by ranitidine partially prevented the kainic acid-induced formation of brain edema, which preferentially occurs in the thalamus (728). In contrast, blockade of H_1 receptors by mepyramine in reasonable dosage did not modify significantly regional cerebral glucose utilization in conscious rats (267).

IX. ROLE OF HISTAMINERGIC NEURONS IN THERMOREGULATION

Several data suggest that HA participates in central temperature regulation. A dose-related hypothermia is observed after either intracerebroventricular injection of the amine (130, 684) or its local application to the rostral hypothalamus (91), a site where some thermoresponsive neurons are also depressed by iontophoretic application of HA (726). The effect of intracerebroventricular HA is potentiated by pretreatment with amodiaquin, an inhibitor of HA methylation (141). *tele*-Methylhistamine, the main HA catabolite in brain, is ineffective when given intracerebroventricularly (141, 441). Loading mice with His in doses able to double brain HA level has no effect on temperature (140), but a similar treatment induces a fall in core temperature of rats (260), and this response is suppressed by blockade of HA synthesis (144). Burimamide, metiamide, or cimetidine, when centrally administered, antagonizes the hypothermia induced by His, 4-methylhistamine, dimaprit, or impromidine (143, 260, 515, 566). These data strongly suggest that H_2 receptors in brain mediate the hypothermic action of HA.

On the other hand, the hypothermia produced by the local application of HA into the hypothalamus was also prevented by a systemic injection of an H_1-receptor antagonist (91). Biphasic changes in the body temperature have been reported in cats after intracerebroventricular administration of HA (130). The immediate fall in temperature is followed by a slight hyperthermia occurring over a number of hours after the injection. Whereas mepyramine, a H_1 antagonist, blocks the HA-induced hypothermia, the delayed hyperthermia, which is mimicked by 4-methylhistamine, is suppressed by pretreatment with metiamide (130). To reconcile these findings it has been suggested (145) that HA affects the central thermoregulatory pathways by at least two mechanisms: stimulation of H_1 receptors located in the rostral hypothalamic thermoregulatory centers may lower the thermoregulatory set point, and stimulation of H_2 receptors, located elsewhere, may somehow activate efferent heat loss pathways directly.

The involvement of histaminergic systems in the ·mal response to phencyclidine has also been pro-ed more recently (358). However, in anesthetized ., stimulation of H_2 receptors in the nucleus preopti-medialis elicits a rise in core temperature (137).

Ionizing radiations in guinea pigs induce hypother-, an effect mediated by both H_1 and H_2 receptors ·). Inhibition of HA synthesis by α-FMH did not mod-ody temperature in rats (83).

:OLE OF HISTAMINERGIC NEURONS IN EMESIS .ND MOTION SICKNESS

Emesis is observed in dogs receiving HA intracere-·entricularly in high dosages, and the response is ·ented by surgical ablation of the chemoreceptor ger zone or by administration of H_1- and H_2-receptor ıgonists (69, 70). The area postrema contains rather ı levels of HDC activity in humans (657) and H_1 re-ors in rodents (86, 414, 543).

Several H_1 antagonists are currently used to pre-: motion sickness in humans, but their efficacy may ve from an associated antimuscarinic activity (188). ertheless, a possible role of endogenous HA is sug-ed by the presence of HA terminals and H_1 recep-in brain stem nuclei, such as the nucleus of the ·ary tract or vestibular nuclei (7, 86) known to be ·lved in motion sickness.

On a behavioral model of motion sickness in rats, kaolin intake elicited by rotation, α-FMH had a ben-al effect; in addition, rotation induced a rise in n stem HA that was prevented by labyrinthec-y (736).

ROLE OF HISTAMINERGIC NEURONS IN CONTROL OF NOCICEPTIVE RESPONSES

A possible role of histaminergic neurons in the CNS ıe control of pain has been recently reviewed (328, 627).

Histamine administered intracerebroventricularly ıts (249) or mice (128, 526) impairs several nocicep-responses, e.g., in the writhing or hot plate jump s, apparently via activation of H_2 receptors. A simi-·esponse is also observed after injections of HA onto ·ons of the dorsal raphe (249) that are depressed); however, in contrast, this response apparently in-es H_2 receptors. A control of pain perception by HA ·ons would be consistent with their projections to the presence of H_1 (86) and H_2 receptors (624a; M. L. thenet, J. C. Schwartz, and M. Ruat, unpublished rvations) in areas, e.g., the external of layers of the al horn of the spinal cord and mesencephalic peri-ricular grey matter, known to be involved in noci-ive controls (67). However, the numerous studies on effects on H_1-receptor antagonists administered e do not clarify this issue (627). Several (but not all) pounds of this class display, like HA itself, antinoci-ive activity in some tests involving supraspinal loci, reas they are consistently ineffective in the tail flick onse considered to be mainly controlled at the spi-nal level. In addition, many of these compounds block not only H_1 receptors but also muscarinic or serotoninergic receptors and the monoamine uptake systems. The fact that both the H_2 agonists and antagonists in high dosages (intracerebroventricularly) elicit antinociceptive responses does not clarify the issue. Thioperamide, a H_3 antagonist, does not modify a variety of nociceptive responses in mice (J. Costentin, personal communication).

A role of brain HA in a form of stress-induced antinociception has been postulated from the inhibition produced by the HDC inhibitor α-FMH or the H_1-antagonist diphenhydramine, whereas cimetidine was ineffective (434). However, others found cimetidine and other H_2 antagonists including zolantidine, a brain-penetrating compound, effective, whereas the H_1 antagonist chlorpheniramine was not (250, 328, 331).

Morphine does not affect HA or *t*-MeHA levels (340, 504) but increases HA turnover in mouse brain (504). However, a role of brain HA in morphine analgesia was not substantiated by the effects of receptor antagonists and a HDC inhibitor (340).

XII. ROLE OF HISTAMINERGIC NEURONS IN CONTROL OF VIGILANCE, SLEEP, AND WAKEFULNESS

A large body of experimental evidence now supports the hypothesis (577, 655) that histaminergic neuronal systems in mammalian brain play an important role in arousal.

Intracerebroventricular administration of HA causes electroencephalogram desynchronization in rabbits, and this action is antagonized by mepyramine (471, 824). Intracerebroventricular administration of 2-thiazolylethylamine, a relatively specific H_1 agonist but not that of dimaprit, a H_2 agonist, increases wakefulness at the expense of slow wave and paradoxical sleep in rats (polygraphic recordings), and this effect is antagonized by systemic mepyramine in low dosage (472). Histamine injected into the ventrolateral posterior hypothalamus elicits a similar effect in cats, which is also antagonized by mepyramine administered locally or systemically in low dosage (436, 437). In this last study it was verified that sodium nitrite, a potent vasodilator, has no similar effect, suggesting that the action of HA is not indirectly due to its local vascular effects. These clear-cut data indicate that stimulation of H_1 receptors in this area where they were visualized by autoradiography (86) mediates arousal in various mammalians. Because H_1 receptors do not appear to control either HA synthesis and release in vitro (25, 28) or the activity of histaminergic neurons in vivo (239, 570) and because short HA axons were visualized in this area (7), it appears that this arousal results from stimulation of postsynaptic H_1 receptors.

Various experimental approaches have also consistently shown that histaminergic neurons are involved in arousal mechanisms. In cats inhibition of HA synthesis by intraperitoneal α-FMH significantly increases deep slow-wave sleep and decreases wakefulness without modifying light slow-wave sleep and paradoxical sleep;

the drug has a similar effect when injected into the ventrolateral posterior hypothalamus (436, 437). The inhibitor administered systemically to rats has essentially similar effects; however, although in one study it was shown to occur during the "dark" phase (395, 793), in another study it mainly occurred during the "light" phase (473).

Conversely, inhibition of HA degradation via local injection of a HMT inhibitor to the ventrolateral posterior hypothalamus selectively increases wakefulness in cats (436, 437).

Arousal is also observed in cats after systemic administration of thioperamide, a H_3 antagonist, and to be blocked by administration of either *R*-α-MeHA, a H_3 agonist, or mepyramine, a H_1-receptor antagonist (437a). These effects suggest that modulation of endogenous HA release, via presynaptic H_3 receptors, indirectly affects arousal mechanisms controlled by postsynaptic H_1 receptors.

The effects observed after blockade of cerebral H_1 receptors by various antagonists administered alone also support the idea that endogenous HA is critically involved in the control of wakefulness. Systemic administration of mepyramine, one of the most selective H_1 antagonists, in low dosage (1-5 mg/kg) causes a significant dose-dependent increase in slow-wave sleep at the expense of wakefulness and paradoxical sleep in rats (472), dogs (808, 809), and cats (437). A similar effect is also observed in rats treated with diphenhydramine (394). In humans, mepyramine was recently shown not to affect the number of awakenings during sleep or the total sleep time (498). However, there is an abundant literature showing that many H_1 antagonists administered to humans during the day produce marked drowsiness, increase the tendency to sleep, decrease the sleep latency, and impair various performances (188, 208, 497). These various properties are designated together under the name of sedative properties that most H_1 antagonists share. All these compounds have the ability to occupy cerebral H_1 receptors at low dosages, i.e., in the therapeutic range or below, as judged from the [^{3}H]mepyramine binding test in the living mouse (589, 591). In contrast a number of recently designed compounds, which do not easily occupy cerebral H_1 receptors, as indicated by either the in vivo [^{3}H]mepyramine binding test or the direct measurement of their cerebral level, do not share these sedative properties and are therefore increasingly used in the therapy of allergic diseases (195, 497, 589, 591). Interestingly a variety of psychotropic agents, currently used in therapeutics as either antipsychotics or antidepressants, which display strong affinity for H_1 receptors in vitro (3, 287, 288, 610, 611, 757) and occupy cerebral H_1 receptors when administered at low dosage (589, 591), do share these characteristic sedative properties. Starting from these various observations it was proposed that sedation elicited by these various drugs in humans could be ascribed to blockade of the arousal mechanisms mediated by histaminergic neurons in brain (589, 591, 611, 655, 662). It has been argued that sedation may as well be ascribed to other properties of these compounds, i.e., to their ability to block muscarinic and α_1-adrenergic receptors, or to their local anesthetic activity (334, 696, 753, 780). However, in most cases, the affinity of these drugs for these other targets is much lower than for H_1 receptors so that only the latter are likely to be occupied in brain at low therapeutic dosage: for instance the K_i of mepyramine for H_1 receptors is at least 1,000-fold lower than for muscarinic receptors (420). In addition, the high potency toward H_1 receptors is the sole common property of these various sedative compounds.

The critical role of histaminergic neurons in arousal mechanisms is not only supported by pharmacological studies but also by the observations that bilateral damage or experimental lesioning of the posterior hypothalamus is accompanied by a state of somnolence or hypersomnia in humans (791a), monkeys (597), rats (464, 488), and cats (484, 485, 727). Furthermore, lesions of the ventrolateral posterior hypothalamus induced in cats by the neurotoxin ibotenic acid specifically aimed at destroying HA perikarya were followed during the next days by large decreases in wakefulness at the benefit of paradoxical and then of slow-wave sleep; after 3 days the animals recovered their control level of sleep, suggesting the intervention of compensatory mechanisms (638).

Cortically projecting HA neurons of rat tuberomammillary nucleus share with other aminergic neurons believed to control states of sleep and wakefulness a number of electrophysiological properties evidenced by extracellular recordings (604). They are firing spontaneously, slowly (2.0 ± 1.5 Hz) and with a regular pattern; their action potentials are of long duration (2-6 ms), with a pattern suggesting the existence of an electrotonic delay between spontaneously generated initial segment and somatodendritic spikes; and corresponding axons display low conduction velocity (0.3-0.5 m/s) consistent with the ultrastructural observations that a large fractions of HA axons are unmyelinated.

Both neurochemical and electrophysiological studies indicate that the activity of histaminergic neurons is maximal during periods of wakefulness. In several rat and guinea pig brain areas, particularly the hypothalamus, the HA level shows a reliable circadian rhythm with the minimum in the dark phase, during which rodents are the more active (529, 660, 777, 793); although levels of His and HDC were not modified, synthesis of [^{3}H]HA in hypothalamic slices was significantly higher when rats were killed during the dark phase (660). Because decreased amine levels and increased synthesis rates are usually associated with enhanced neuronal activity, it was postulated that this was also the case for histaminergic neurons and that these observations were taken as supporting their role in arousal mechanisms (660).

In the freely moving cat, single-unit extracellular recordings in the ventrolateral posterior hypothalamus detected two populations of neurons, the activity of which was strictly related to cortical desynchronization (784). One of them, which displayed electrophysiological properties very similar to HA neurons recorded in the tuberomammillary nucleus of the anesthetized rat

04), was highly selective for the waking state. During tive wakening these neurons discharged slowly and gularly, the firing rate then became progressively owed during "calm wakening" and, even more, light ow-wave sleep; these neurons were completely silent ring deep slow-wave or paradoxical sleep (784). Barbirates and other hypnotics dramatically decrease HA rnover in brain (327, 571).

Hence a variety of complementary experimental proaches indicate that HA neurons exert a major ntrol in wakening mechanims, their best substanated functional role so far. Available experimental evince suggests that this action is mainly subserved via $_1$ receptors, but the role of H_2 receptors remains to be oroughly investigated. It has been underlined that the sentially modulatory mode of action of HA mediated H_2 receptors at the cellular level and the anatomic sposition of HA pathways fit particularly well with a le in the regulation of states of awareness (276, 577). nally, H_3 receptors may also participate by controlng not only the activity of HA neurons but also that of her neuronal systems.

II. ROLE OF HISTAMINERGIC NEURONS IN CONTROL OF VARIOUS BEHAVIORS

Relatively little research has been centered on the le of histaminergic systems in the control of complex haviors (for review see Ref. 816).

Stress

Contradictory results have been reported regarding e effects of various stressful procedures in rats and ice.

In rats, forced immobilization and/or cold exposure creased HA levels and increased HA turnover in hypoalamus (742, 744), but the effect on HA levels could t be confirmed by others (408). Air blasts increased A levels and HDC activity in rats (461, 463).

In mice, forced immobilization for a relatively short riod reduced whole brain HA turnover without afcting HA levels (789), whereas the same treatment for veral hours decreased HA levels (745). Footshock ress for 30-120 min did not affect HA levels but enanced HA turnover, an effect partly mediated by enogenous opioids (835).

Motor Activity

Intracerebroventricular administration of HA icits biphasic changes in spontaneous locomotor activy in fowl (509), rats (373), and goats (774), with the yperactivity response being, in all cases, mediated by $_1$ receptors. However, in other studies only hypoacvity was observed (110, 525). Inhibition of locomotion, parently mediated by H_1 and H_2 receptors, was obrved in rats shortly after injection of HA into the ventral hippocampus (14). Intra-accumbens injection of HA in rats elicited an initial hypoactivity, apparently mediated by H_3 receptors, followed by hyperactivity, apparently mediated by H_1 receptors (93, 94).

In mice, histidine loads reduce the amphetamine-induced locomotor activity (142, 355), an effect that might involve endogenous HA, as it is suppressed by administration of α-FMH (355).

In humans, several H_1 antagonists known to cross the blood-brain barrier impair psychomotor performance evaluated in a variety of complex skilled tasks (for review see Ref. 816).

C. Drinking and Eating

A role for peripheral HA in the control of peripprandial drinking has been proposed (415) but is not considered here. When injected into several hypothalamic areas, particularly the medial rostral areas, HA elicits drinking in water-satiated rats and increases drinking in water-deprived rats (247, 430, 431). These effects appear to involve both H_1 and H_2 receptors, but blockade of these receptors by peripherally administered antagonists does not result in clear-cut effects on drinking behavior (415-417, 431).

When injected intracerebroventricularly, HA suppresses food intake in cats, an effect possibly mediated by H_1 receptors (133). However, in rats, when the amine is injected into the hypothalamus (136) or continuously infused into the suprachiasmatic nucleus (793), it has an opposite effect.

A possible role of endogenous HA in the control of food intake is suggested by the observation that H_1 but not H_2 antagonists induce feeding in rats when infused into the ventromedial hypothalamus during the light phase of the day; this effect was prevented by pretreatment with α-FMH (527a, 634, 635). Histidine loads (685) or thioperamide (T. K. Sakata, personal communication) suppress food intake.

D. Aggressive Behaviors

Intracerebroventricular administration of HA decreases electric shock-induced fighting in rats (450, 598) but has an opposite action when administered in high doses to mice (486). Histidine loads inhibit the same behavior in mice (142). Various H_1 antagonists, generally in high dosage, suppress isolation-induced fighting in mice (46) or muricide activity in rats (527).

E. Self-Stimulation, Reinforcement, and Aversion

Histamine injected into rat hypothalamic perifornical area, where an electrode for self-stimulation was implanted, inhibited self-stimulation, and the effect was blocked by H_1 antagonists (136). Mepyramine decreases self-stimulation, but chlorpheniramine has an opposite effect (807).

The H_1 antagonists are self-administered intravenously in squirrel monkeys (63) but not orally in rats (222).

When intracerebroventricular administration of HA was paired with administration of a sucrose solution, taste aversion to the later developed (596).

F. Learning

Intracerebroventricular administration of HA to rats improved the retention of a learned behavior, apparently via stimulation of both H_1 and H_2 receptors (161).

G. Discriminative Properties of H_1 Antagonists

In various animals trained to discriminate a H_1 antagonist from saline, there is complete generalization to most other drugs of this class (533, 815, 823). This suggests that the H_1 antagonists share some common behavioral properties but does not clarify the nature of the latter.

H. Hallucinatory Properties of H_2 Antagonists

There are several reports indicating that in a small number of patients taking cimetidine or ranitidine visual or auditory hallucinations may occur, perhaps as a result of altered blood-brain barrier permeability (for review see Ref. 816). It is not clear, however, whether these effects are selectively related to H_2 receptor blockade.

XIV. CONCLUSION

During the last few decades, many reviews on the physiological role of HA in the brain were almost uniformly concluded by the assertion that the amine should be considered as a putative neurotransmitter. The data reviewed here demonstrate, beyond any reasonable doubt, that this role is now fully established. Histaminergic neurons, although in small number, constitute one of these long tracts with widespread projections to large CNS areas, particularly in diencephalon and telencephalon. The localization of their target cells and the modes of action of HA at their level, as it is mediated by the three receptor subtypes, seems also largely, although incompletely, unraveled. From the available information it has been for a long time suspected that histaminergic neurons may coordinate diverse sensory, motor, hormonal, and vegetative functions to act in a concerted manner. These rather vague functions are similar to those currently ascribed to other aminergic neurons (noradrenergic, serotoninergic), but the picture is somewhat less clear in the case of HA. One of the major challenges to take up now seems to be the understanding of the exact functional role of histaminergic neurons. Their implication in a variety of biological and physiological processes (e.g., arousal, pituitary hormone secretion, control of cerebral circulation, thermoregulation) has been so far mainly suggested from the observed actions of HA or, in few cases, of agents modifying histaminergic transmissions in the brain. However, in most cases, one notable exception being arousal: there is a lack of evidence that these physiological activities are correlated with changes in neuronal histaminergic activity.

Monitoring these changes in behaving animals seems a prerequisite to more firmly confirm these various contentions. Establishing the nature of the information received by histaminergic neurons seems equally important in this respect. Also, as in the case of most other neuronal populations, the functional significance of coexisting neurotransmitters, which are particularly numerous in histaminergic neurons, needs to be established. In addition there is, so far, no clear indication regarding a possible implication of histaminergic neurons in pathophysiology.

Finally, a major challenge that remains to be taken up is the understanding of the functions of the nonneuronal store(s) of HA in the CNS.

The dramatic progress recorded during the last few years in understanding the other functions of the amine make us confident that little time will elapse before these remaining and exciting physiological problems are solved.

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Histaminergic Transmission in the Mammalian Brain

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I. INTRODUCTION

The recognition of histamine (HA) [or 2-(4-imidazolyl)ethylamine] as a messenger molecule in cell-to-cell communication began early in this century. The early history of HA was dominated by Sir Henry Dale, the great British pharmacologist; Barger and Dale (44) were the first to identify the amine in ergot extracts. Thereafter Dale and Laidlaw (151, 152) described the major actions of HA on tissues, i.e., its potent contractile effects on smooth muscles and the capillary dilative action. Popielski (579) described the only other important HA effect, the stimulant effect on gastric secretion.

In 1927, Dale and co-workers (68) were among the first to isolate HA from a variety of fresh tissues, thus establishing that HA is a normal constituent of the body. In fact its name derives from "histos," the Greek word for tissue. However, Dale was reluctant to accept the notion that endogenous HA could function as a messenger molecule, that is, that HA could be released from its tissue stores to affect the activity of target cells. It was Feldberg (211, 212) who clearly demonstrated that HA was released from the lung during the anaphylactic response and that it induced a marked bronchoconstriction.

These experiments suggested the role of HA in the mast cells of connective tissue, which are only one of its major stores. Although HA has long been suspected of not being restricted to mast cells, there was little knowledge of the localization and functions of the non-mast cells stores of the amine until recently.

Insights on possible HA functions in the central nervous system (CNS) have been much slower in arriving than studies on other amines. Nevertheless, the presence of HA in brain can be traced back as far as other biogenic amines (1). Kwiatkowski (426) found that HA was more concentrated in gray matter than in white matter, and White (817) first showed that local synthesis of the amine occurs in the brain.

It was not until 1970 that interest in brain HA resumed when sensitive radioisotopic assays for the amine and for L-histidine decarboxylase (HDC), its synthetizing enzyme, were developed (667, 743). These assays enabled the rapid accumulation of data, mainly biochemical, that indicated that HA needed to be added to the already long list of neurotransmitters. In particular, lesion studies performed by Garbarg et al. (232) demonstrated for the first time the existence of histaminergic neurons in mammalian brain. This was confirmed when immunohistochemical tools to detect the amine (552, 707, 821) or its synthetizing enzyme (802) became available, and the origin and projections of these neurons are fairly well established now. Meanwhile it had been established that cerebral HA could be released by depolarization and in a calcium-dependent manner (32) and that its turnover is rapid and can be modified almost instantaneously (568, 569).

Equally important in establishing the role of HA as a neurotransmitter was the demonstration of the presence in brain of three subclasses of receptors by biochemical (25, 56, 618) and electrophysiological approaches (281).

Thus the two last decades of research have resulted in a coherent picture of the involvement of HA in neuronal communication. In addition, the design of an increasing number of pharmacological tools to selectively modify HA transmission and their utilization in electrophysiological, neurochemical, behavioral, and neuroendocrinological studies has led to a progressively more precise hypothesis about the functions of HA neurons in the CNS.

The topic of HA in mammalian brain has been the subject of various general reviews in the last few years (228, 277, 328, 577, 584, 615, 666). Specific HA neurons and their targets have also been identified in the CNS of the invertebrate mollusk *Aplysia*, but this topic is not considered here (for review see Ref. 811). The topics of brain HA receptors (105, 137a, 227, 255, 256, 304, 330, 659) and of methodology in brain HA research have also been reviewed (238, 786, 805).

II. METABOLISM OF HISTAMINE

A. Biosynthesis

Histamine poorly penetrates the brain from blood (648, 671) and therefore must be formed locally. Indeed, in vivo formation of radioactive histamine in brain was detected after administration of its radioactive precursor L-histidine (His) in cats (818), rats (571), or mice (789). Histamine biosynthesis involves two steps, i.e., transport of His into the cell and its decarboxylation by HDC (for reviews see Refs. 33, 238, 576, 805).

1. Histidine transport

Saturable, energy-dependent His uptake occurs in brain slices (788) and synaptosomes (127, 299, 365). The transport system in synaptosomes seems partly independent of Na^+ and K^+, and kinetic analyses revealed the presence of both high- and low-affinity components. Histidine uptake is stimulated by depolarization (299, 788). There is not yet evidence for the presence of a specific His transport system in HA neurons, since amino acid transport is not altered after lesions of the latter (299). However, detection of such a system might be difficult in view of the small number of HA nerve endings in brain. The His level is similar in the C_2 histaminergic and nonhistaminergic neurons of *Aplysia* (813).

2. Properties of L-histidine decarboxylase

L-Histidine decarboxylase (EC 4.1.1.22) and dopa decarboxylase (EC 4.1.1.28) is responsible for the one-step HA formation in brain (42, 650, 651, 667, 813). L-Histidine decarboxylase has been exclusively purified from various peripheral tissues displaying high

catalytic activity (268, 292, 454, 575, 576, 642, 643, 731, 758, 801, 806, 832) and from most studies appears as a protein of 110-125 kDa constituted of two identical subunits of 55-60 kDa with an isolectric point around 5.5. The existence of various HDC isoenzymes has been suggested from isoelectric focusing patterns (454, 642, 643) and immunologic studies (804, 805). In addition, variation in the structure of kidney HDC in mouse strains affecting its affinity for its cofactor and its heat stability was shown and attributed to the existence of alleles of a single structural gene on chromosome 2 (455).

However, HDC from various rat tissues, including the brain, displays similar catalytic activity and is similarly recognized by both monoclonal (573-575) and polyclonal antibodies (731). Also HDCs in brain homogenates from *Aplysia californica* (813), rat (667), mouse (745), guinea pig (650, 651), rabbit (516), hamster (296), and human (42) display rather similar catalytic activity. Similar to other mammalian decarboxylases, HDC functions with the cofactor pyridoxal-5'-phosphate, which is so highly bound to the apoenzyme (456, 540, 541) that its addition to cerebral extracts does not result in marked increases in catalytic activity (Schwartz, Garbarg, and Pollard, unpublished observations). Characteristically, HDC decarboxylates His with a Michaelis constant (K_m) and a maximum rate (V_{max}) that change with the pH and ionic strength of the medium (33, 290, 667, 805). At a pH of ~7.0 in standard buffers the K_m of His is ~0.1 mM, a value that appears to be close to the plasma concentration and, presumably, the intraneuronal concentration of the amino acid, accounting for the observations that HA levels in rodent brain are increased after systemic administration of His in large dosages (668, 745). The turnover number of purified HDC (~0.4 s^{-1}) seems low compared with that of other amino acid decarboxylases (454).

Recently, the complete amino acid sequence of rat HDC was deduced from the cloning, from a fetal liver library, of a cDNA encoding the enzyme (367a). It comprises 655 amino acid residues, corresponding to a protein of 73 kDa, a value significantly higher than the 55- to 60-kDa values found after purification of the subunits, suggesting that HDC might be postranslationally processed. Rat HDC displays distinct homologies with other pyridoxal phosphate-dependent enzymes, such as dopa decarboxylase, particularly in the region surrounding the putative cofactor-binding lysine residue (Lys^{307}). The sequence comprises two consensus phosphorylation sites of cAMP-dependent protein kinase. The HDC gene was located to mouse chromosome 2, where it is closely linked to the β_2-microglobulin gene (367a).

L-Histidine decarboxylase displays a high substrate specificity toward His, with the sole other decarboxylated natural amino acid being 3-methylhistidine, which yields *tele*-methylhistamine (*t*-MeHA) (674), a substance with little biological activity that is mainly formed in brain by methylation of HA. Because both the affinity of 3-methylhistidine for HDC and its tissue levels are low, the functional significance of this minor pathway remains doubtful. Other natural amino acids display even lower apparent affinities for HDC, and their decarboxylation was not demonstrated (633). The two HDC inhibitors α-methylhistidine (194, 650) and α-fluoromethylhistidine (412) are decarboxylated by HDC.

In several peripheral tissues, such as the stomach or skin, HDC activity can be rapidly and markedly increased as a result of various treatments, e.g., by hormones (647), that may induce synthesis of HDC molecules (673). In brain, similar changes were not reported but, after its nearly total inactivation by a "suicide-inhibitor," cerebral HDC is synthesized at a high rate: ~50% of the initial HDC activity is recovered within 26 h in the hypothalamus, where HA perikarya are located, and within 58 h in the cerebral cortex, which contains nerve endings (235). From this time lag it has been inferred that HDC is transported at a rate of ~1 mm/h, roughly corresponding to the mean transport rate of other monoamine-synthesizing enzymes.

3. *Localization of L-histidine decarboxylase*

Biochemical and immunohistochemical studies have revealed the presence of HDC in the cytoplasm of a population of cerebral neurons (see sect. III). L-Histidine decarboxylase activity is distributed in a markedly heterogeneous fashion between cerebral regions, with the highest levels being found in hypothalamus, the lowest levels in cerebellum, and intermediate levels in telencephalic areas (55, 664, 667, 745). Heterogeneous distributions are also found among nuclei of the hypothalamus (567), upper brain stem (572), amygdaloid complex (60), or areas of the hippocampal formation (40). In contrast, HDC activity does not vary markedly between areas of the cerebral cortex (42, 231). These regional distributions of HDC activity are generally consistent with data derived from immunohistochemical studies (see sect. III).

Subcellular fractionation studies indicate that HDC is mainly found in the cytoplasm of isolated nerve endings (55, 745, 751), a conclusion confirmed by immunohistochemical studies at the electron-microscopic level (294). In the hypothalamus, which contains HA perikarya, a lesser proportion of HDC activity than in other areas is associated with subcellular fractions enriched in nerve endings (55), and HDC immunoreactivity is detected all over the cytoplasm surrounding the large cell nucleus of these neurons (294). In the neonatal brain, i.e., before formation of most HA synapses, HDC activity is low, particularly in telencephalic areas, and mainly recovered in soluble (nonsynaptosomal) subcellular fractions (457, 751). The earliest detection of HDC in hypothalamus during postnatal development is consistent with the expression of the enzyme in perikarya from this region (457).

Although HA neurons constitute the major localization of HDC, small fractions of the cerebral enzyme are held in mast cells (457).

4. *Inhibition of L-histidine decarboxylase*

Because HA is synthesized in a single step and by a highly specific enzyme, HDC inhibitors are potentially

useful tools with which to investigate the role of HA. Until recently, only compounds with limited specificity and low potency, such as α-hydrazinohistidine, brocresine, or α-methylhistidine, were available (433, 667, 668, 743, 745). In 1978, Kollonitsch et al. (412) designed *S*-α-fluoromethylhistidine (α-FMH) among a series of potential "suicide" or catalytic constant (K_{cat}) inhibitors of amino acid decarboxylases. *S*-α-fluoromethylhistidine potently inhibits in a stereoselective, time-dependent, concentration-dependent, and irreversible manner cerebral HDC with an inhibitor constant (K_i) of $\sim 10^{-5}$ M, whereas related decarboxylases such as dopa or glutamate decarboxylase are not significantly affected (235). Indeed α-FMH is a suicide substrate that remains bound to the enzyme after being decarboxylated (194, 421). *S*-α-fluoromethylhistidine is similarly potent on HDCs of humans (763), *Aplysia* (812), and even *Morganella morganii* (295).

From this mechanism it seemed feasible to covalently label and detect HDC autoradiographically on tissue sections; however, because of a high background, this procedure could not be applied to histochemistry (665). *S*-α-fluoromethylhistidine, administered systematically in rather low dosages, rapidly, completely, and in a long-lasting manner inactivates HDC in brain and peripheral organs (82, 83, 235, 296, 446, 692, 792). Restoration of activity occurs progressively, presumably as a result of neosynthesis of HDC molecules, and in brain the process is first detected in the hypothalamus in which HA perikarya are located (235). Because α-FMH efficiently depletes HA stores in cerebral neurons (235, 446), the drug is a useful tool for investigating the amine turnover and functions therein.

5. *Regulation of histamine biosynthesis*

Various treatments that drastically affect HA turnover in brain fail to alter its steady-state level to any large extent, implying the existence of efficient regulatory processes (356, 357, 360, 504, 571). The latter can even be demonstrated to occur in vitro, since the depolarization-induced release of [^{3}H]HA from slices or synaptosomes incubated in the presence of [^{3}H]His is accompanied by a large increase in the rate of [^{3}H]HA formation (23, 26, 28, 787, 788). In the absence of Ca^{2+} in the external medium, not only release but also stimulation of synthesis is abolished, suggesting some relationships between the two effects. However, this interpretation is not entirely clear, since [^{3}H]HA synthesis is already activated by removal of external Ca^{2+} in the absence of depolarization (28).

The mechanism by which external Ca^{2+} regulates [^{3}H]HA synthesis is not known, but parallel changes in catecholamine formation have been ascribed to activation of the rate-limiting enzyme tyrosine hydroxylase by phosphorylation via adenosine 3',5'-cyclic monophosphate (cAMP)- or Ca^{2+}-dependent protein kinases (252). A similar process was preliminarily suggested to occur in the case of cerebral HDC, the activity of which was enhanced by preincubation with a cAMP-dependent protein kinase (191), but this could not be confirmed on mastocytoma HDC (291). Theoretically, depolarization-induced stimulation of [^{3}H]HA formation could result from an enhancement of [^{3}H]His uptake, but the latter is only weakly enhanced in slices (788) and even decreased in synaptosomes (127, 299). However, stimulation of a quantitatively minor but specific system for the amino acid uptake in HA neurons having so far escaped detection cannot be discarded. A direct feedback inhibition of soluble HDC by added HA has been excluded (668).

Histamine formation in brain slices and synaptosomes as well as in rat brain in vivo is, similar to [^{3}H]HA release, regulated via H_3 autoreceptors (23, 26, 28, 239). In vitro, K^+-induced stimulation of [^{3}H]HA formation is reduced by 60-70% in the presence of HA or H_3-receptor agonists in low concentrations; conversely, it is enhanced by H_3-receptor antagonists, presumably via blockade of autoinhibition elicited by released HA, acting at the autoreceptors. The mechanism of H_3 receptor mediated regulation of [^{3}H]HA formation is not known mainly because the transduction system of H_3 receptor is not yet identified.

Stimulation of presynaptic M_1 muscarinic (270) or α_2-adrenergic receptors (271) in slices or in rat brain in vivo also reduces [^{3}H]HA formation to the same extent as H_3 receptor stimulation does.

B. *Storage*

Histamine is present in the brain of invertebrates (607), lower vertebrates (10), and mammals. In several mammalian species, such as dogs (4), cats (5), rabbits (254), monkeys (513, 741), guinea pigs (79), rats (619, 743), mice (629, 660), hamsters (296), and humans (440), it is distributed in a highly nonuniform manner between grossly dissected regions and even among nuclei in regions such as the hypothalamus (98, 567).

A variety of biochemical and pharmacological approaches strongly indicated that the cerebral amine was held in at least two classes of cellular stores, neurons and mast cells (654). With the advent of histochemical methods, these two classes of HA-storing cells have now been visualized (see sects. III and IV). Although mast cells are scarce in the brain, because of their high HA content they significantly contribute to the overall amine content, particularly in certain brain regions.

Data from subcellular fractionation studies (123, 231, 383, 423, 457, 467, 654, 697) are consistent with this dual localization. In mast cells from various tissues, HA is held in large granules that sediment with the crude nuclear (P_1) fraction. Consistently a fraction of cerebral HA (~20%) sediments with this fraction, a feature that is not observed for other amines. In addition, a large fraction of the amine sediments with both the crude mitochondrial (P_2) and microsomal (P_3) fractions that both contain synaptosomes, i.e., pinched-off nerve endings. Within the P_2 and P_3 fractions, HA is largely bound to vesicles, whereas HDC is mainly soluble: this indicates that HA, similar to other neurotransmitters, is synthesized in the cytoplasm of nerve endings and

thereafter bound to synaptic vesicles. In the bovine retina, ~80% of HA sediments with the heavy P_1 fraction, which contains vascular elements and large nerve endings (513).

Microvessels isolated from homogenates of bovine (362), rat (382), or guinea pig brain (616) have high levels of HA with low HDC activity. In these fractions HA might be held either in endothelial cells or mast cells that are intimately associated with the cerebral vasculature.

C. Release

1. Release in vitro

Endogenous HA is released by K^+-induced depolarization of cerebral slices via a Ca^{2+}- and temperature-dependent process (32, 360, 505, 519, 747). However, on this model the spontaneous efflux of HA is relatively high, as some part of the amine might originate from mast cells, and the K^+-induced stimulation is of limited amplitude, resulting in only 50-100% increases in the HA level in medium. The 20 mM K^+-induced release of endogenous HA from slices of mouse hypothalamus is enhanced at low concentrations of glucose, an effect largely prevented by tetrodotoxin (505).

Tritiated HA synthesized from [^{3}H]His in slices from various brain areas is released by depolarizations induced by K^+ (25, 27, 28, 787, 788), electrical stimuli (781–783), or veratridine (25, 27). This occurs over a low spontaneous efflux and results in a 5- to 10-fold increase in [^{3}H]HA in medium. Release of [^{3}H]HA from depolarized slices is completely inhibited in the absence of Ca^{2+} or in the presence of 10 mM Mg^{2+}, suggesting that it results from the opening of voltage-sensitive Ca^{2+} channels (25, 782, 788). Doubling the external Ca^{2+} concentration diminishes the 30 mM K^+-induced release of [^{3}H]HA, presumably via inactivation of Ca^{2+} channels by an excess of intracellular Ca^{2+} (27). Conducted action potentials are presumably involved for a small part in the 30 mM K^+-induced release, because the latter is only weakly reduced in the presence of tetrodotoxin (27). Neosynthesized [^{3}H]HA is also released from depolarized synaptosomes (27) but not from mast cells (788). All these features are consistent with the idea that depolarization of histaminergic nerve endings induces HA secretion via mechanisms similar to those operating for other neurotransmitters.

Superfused brain slices preincubated in the presence of [^{3}H]HA accumulate the amine against a small concentration gradient (tissue-to-medium ratio of 2) and release radioactivity on depolarizations induced electrically or by K^+ or veratridine (74, 475, 476, 512, 715, 716). Although the Ca^{2+} dependency of the process suggests that [^{3}H]HA originates from neurons, its significance remains doubtful. Indeed, histaminergic neurons apparently lack selective reuptake systems for HA, and the extent of [^{3}H]HA uptake in slices from various brain regions does not parallel the relative densities of HA axons. Moreover, from the effects of neurotoxin-induced lesions it appears that a fraction of exogenous [^{3}H]HA enters dopaminergic and 5-hydroxytryptamine (5-HT) neurons (695).

Compound 48/80, a mast cell degranulator, releases endogenous HA (788) and exogenous [^{3}H]HA (716) but not neosynthesized [^{3}H]HA (788) from hypothalamic slices. In contrast, reserpine, a drug interfering with storage processes in aminergic neurons, releases both endogenous HA (747) and neosynthesized [^{3}H]HA (787) from these slices. These differential effects presumably illustrate the multicompartmentation of the amine in the brain and the fact that, in contrast with the neuronal pool, the slowly turning over pool is not readily labeled by [^{3}H]His. Mast cells do not possess voltage-dependent Ca^{2+} channels in their plasma membrane and therefore do not release HA on depolarization (187, 716).

2. Release in vivo

Spontaneous and K^+-induced release of endogenous HA into superfusates of the hypothalamus of anesthetized cats and freely moving rabbits was detected (560, 562). The spontaneous release appeared to vary rhythmically in hypothalamus but also in mammillary bodies or medial amygdaloid nucleus (583). After lesions of the ascending histaminergic pathway at the hypothalamic level, the HA content in the cerebral cortex transiently rises, which may reflect an impaired release resulting from the interruption of impulse flow (40, 231). Electrophysiological responses triggered in the cerebral cortex (640), hippocampus (284, 285), or nucleus accumbens (126) by large stimulations of putative afferent pathways were significantly impaired by iontophoretic application of HA receptor antagonists.

These observations may be taken as indirect evidence for a stimulation-induced release of endogenous HA in vivo.

3. Control of histamine release by autoreceptors and heteroreceptors

In slices from several regions of rat brain the release of neosynthesized [^{3}H]HA induced by either K^+ (25–27), veratridine (25–27), or field electrical stimulation (781–783) is strongly inhibited on addition of exogenous HA in the external medium. In overflow experiments, the maximal inhibition of release may be as high as 80% with 30 mM K^+ stimulations (666) or nearly total with electrical stimulation (782, 783). Inhibition is more marked for depolarizing stimuli of low intensity (27). The autoinhibitory effect of HA appears to be a receptor-mediated effect, since it displays saturability [the half-maximal effective concentration (EC_{50}) of HA is ~0.1 μM], reversibility (it is suppressed by washing out the excess of exogenous HA from the preparation), and high pharmacological specificity, being mimicked or antagonized in a competitive manner by several agents displaying low if any activity at H_1 and H_2 receptors.

This last feature has led to the pharmacological definition of H_3 receptors (23, 25). The fact that H_3 receptors are directly located on histaminergic terminals was shown by various data: *1*) the autoinhibitory effect of HA persists when the propagation of action potentials in the brain slice is blocked by tetrodotoxin, *2*) it also persists in slices from kainate-injected striatum, and *3*) it is also found with a synaptosomal preparation (26, 27). The extent of the autoinhibition can be modulated in a complex manner by changes in extracellular Ca^{2+}, suggesting that H_3 receptors regulate HA release via a control of Ca^{2+} entry (27).

The fact that selective H_3-receptor antagonists modestly but significantly enhance [^{3}H]HA release from depolarized slices and, when systematically administered, markedly enhance cerebral HA turnover indicates that H_3 receptors are tonically activated in vivo (23, 239, 519a). This observation might be related to the fact that endogenous HA concentration in human lumbar cerebrospinal fluid (CSF) ($\sim$0.4 μM) is above the EC_{50} of HA at H_3 receptors (34, 388, 714). However, HA levels were found to be clearly lower when assayed by different methods (776a), and HA levels in ventricular CSF or extracellular fluid of the brain are not known.

Release of neosynthesized [^{3}H]HA from rat brain slices and synaptosomes is inhibited by stimulation of muscarinic M_1 receptors (270), α_2-adrenoreceptors (271, 310, 312), and opioid κ-receptors (271a). In contrast, [D-Ala^2-*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAGO), a specific agonist at μ-opiate receptors stimulates the 30 mM K^+-evoked HA release from mouse brain slices (360). Hence not only auto- but also heteroreceptors appear to control the amine release.

D. Inactivation

1. Uptake

In several studies with brain slices or synaptosomes, no evidence for an active transport system for HA operating at histaminergic nerve endings could be found (324, 489, 664, 740). In contrast, uptake of labeled HA in brain slices, occurring against a limited concentration gradient, was reported in other studies (74, 475, 476, 512, 716, 778), some of which also demonstrated that the radioactivity could be subsequently released by depolarization (see sect. II*C*). However, the maximum tissue-to-medium ratio was low, the energy dependence of the process not demonstrated, the regional distribution of this accumulation did not reflect that of HA neuronal markers, and finally release of accumulated [^{3}H]HA was not regulated by H_3 autoreceptors. This suggests that no significant uptake occurred at HA nerve endings. On the contrary, recent lesion studies indicate that a fraction of [^{3}H]HA is accumulated into serotoninergic and dopaminergic neurons (695). In summary, HA neurons appear to be almost unique among monoaminergic neurons in that they lack a high-affinity reuptake system.

2. Transmethylation of histamine

In various tissues, HA catabolism occurs along two alternative pathways, i.e., transmethylation into *t*-MeHA catalyzed by histamine *N*-methyltransferase (HMT, EC 2.1.1.8) and oxidative deamination into imidazolacetic acid, catalyzed by diamine oxidase (histaminase, EC 1.4.3.6) (602, 603, 645). Contrary to an early report (698), only the former pathway operates in mammalian brain, as shown by the failure to detect diamine oxidase activity (103) or formation of labeled imidazolacetic acid after intracerebral administration of labeled HA; in contrast, both *t*-MeHA and its deaminated metabolite *tele*-methylimidazole acetic acid (*t*-MIAA) were detected (648, 649, 671). Low levels of *t*-MeHA are present in monkey and human CSF (389, 585, 722, 723).

Methylation also appears to be the main, presumably the only, pathway for the catabolism of endogenously synthesized [^{3}H]HA in vivo (571, 601, 602, 648, 649, 672, 818) or in vitro after its depolarization-induced release (27, 788). Endogenous *t*-MeHA is present in brain at levels in the same range as those of HA and with a similar regional distribution (238a, 330, 336, 521, 522, 629). The large decreases in *t*-MeHA levels elicited by inhibition of either HDC (521, 522, 629) or HMT (339) imply that in vivo *t*-MeHA is mainly formed by HA methylation. However, a small contribution of the pathway involving decarboxylation of the natural amino acid *tele*-methylhistidine by HDC cannot be dismissed (674). Subcellular fractionation indicates that, in contrast to HA, *t*-MeHA is more abundant in the supernatant than in synaptosome-containing fractions (336), suggesting its predominantly extraneuronal formation.

tele-Methylhistamine is inactive at H_1, H_2 (226), and H_3 receptors (25). The activity of HMT is high in the brain of various mammalian species in which its rather homogeneous regional distributions does not reflect that of HA neuron markers (36, 96, 628, 664, 771, 777). On subcellular fractionation, cerebral HMT is mainly recovered in the supernatant, with a rather minor contribution of the soluble fraction from synaptosomes (95, 423). Histamine *N*-methyltransferase is present in both glioma and neuroblastoma cell lines (236) as well as in cerebral microvessels (382). Neither lesion-induced degeneration of HA neurons nor intrahippocampal kainate (234) significantly affect telencephalic HMT (75, 237). In contrast, HMT was reduced in the striatum after local administration of kainate (699) and in the neurohypophysis after section of the pituitary stalk (785). Although HMT has not been visualized histochemically, all these data suggest that HMT is present in the cytoplasm of a variety of cerebral cells into which released HA has to enter (see sect. II*D1*). Diurnal (777) and hypertonic saline-induced (785) variations in neurohypophyseal HMT have been described.

Histamine *N*-methyltransferase from various tissues has been purified to homogeneity (88, 293, 460) and its properties reviewed (81, 786). The enzymes from brain and kidney have similar physicochemical, catalytic, and immunologic properties. They have a M_r of 30,000, a pI of 5.3, and an optimum pH for HA methylation of 7.5–9.

They catalyze the transfer of a methyl group from the universal donor *S*-adenosyl-L-methionine selectively to the *tele*-nitrogen of the imidazole ring of HA. The K_ms of both substrates are ~10 μM. No endogenous substrate other than HA has been identified. In vitro, HMT is inhibited by HA at concentrations > 100 μM (47, 460, 746), but this may not have physiological relevance. Histamine *N*-methyltransferase is also inhibited by its two reaction products, i.e., *t*-MeHA (96, 438) and *S*-adenosyl-L-homocysteine (53, 529, 664, 837), with K_is in the same range as the K_ms of its two substrates, implying a possible regulatory role (664). Although a different kinetic mechanism was proposed (748), a variety of features indicate that HMT functions with an ordered bi-bi-mechanism (30, 53, 219, 530).

In vitro, HMT can be inhibited by a large variety of drugs including H_1-, H_2-, and H_3-receptor antagonists (for review see Ref. 786), with one of the most potent compounds being SKF 91488, a dimaprit analogue devoid of activity at receptors (58). In vivo, the most effective compounds to inhibit cerebral HMT are amodiaquin (664, 748) and, even more, metoprine (192). The latter decreases *t*-MeHA (339, 357, 835) and increases HA in rat brain (192, 838), confirming the key role of HMT in HA inactivation.

3. Oxidative deamination of tele-methylhistamine

The cerebral levels of radioactive *t*-MeHA formed from either labeled HA (648, 649, 671) or labeled His (571, 601) rise markedly in rodents treated with monoamine oxidase (MAO) inhibitors, such as pargyline, that are devoid of diamine oxidase inhibitory capacity. These drugs also increase endogenous *t*-MeHA (238a, 338, 522). This occurs without marked changes in levels of endogenous or labeled HA. Hence MAO seems responsible for the oxidative deamination of *t*-MeHA. The type B isoenzyme of MAO catalyzes the reaction, as shown with MAO inhibitors more selective than pargyline (329, 796).

Deamination of *t*-MeHA results in the formation of *t*-MIAA (645), which therefore represents the final product of HA metabolism in mammalian brain (389, 648, 649). Its level is reduced, although slowly, after inhibition of either HMT (339) or HDC (721). Probenecid, a drug inhibiting the active transport of deaminated metabolites of various monoamines from brain, does not apparently affect that of *t*-MIAA (387, 671). Endogenous *t*-MIAA is present in monkey and human CSF at levels slightly higher than those of *t*-MeHA (389, 585, 723).

4. Other inactivation pathways

The good agreement between turnover rates derived from the application of various methods indicates that formation of *t*-MeHA and its subsequent deamination constitutes the major if not the sole metabolic pathway for cerebral HA (see next section). Although mammalian brain contains an *N*-acetylhistamine deacetylase (205, 297), endogenous acetylation of HA is not substantiated.

In invertebrates a major inactivation pathway for HA may consist of the formation of the peptidoamines γ-glutamyl-HA (703) or α-alanyl-HA, i.e., carcinine (20). Peptidoamines are also formed when HA and various amino acids are incubated with brain homogenates, but the physiological relevance of this observation has remained obscure (413, 599).

E. Turnover

Isotopic tracer methods, the first to be applied to the determination of HA turnover rate in brain, revealed that the half-life of the amine is a matter of minutes and can be altered almost instantaneously (170, 571). These early observations, which supported the contention that HA was released from neurons, have been essentially confirmed and extended with the development of various nonisotopic methods (for reviews see Refs. 258, 629).

1. Isotopic methods

With no selective uptake mechanism to mix radioactive HA to the endogenous stores, the latter must be labeled with the radioactive precursor to realize turnover studies. In early studies, [^{3}H]His was administered intracerebroventricularly in lightly anesthetized rats, and the fluctuations of the specific radioactivities of [^{3}H]HA and [^{3}H]His were typical for a precursor-product relationship in a single open compartiment (170, 571). Analysis of this relationship using two different mathematical models led to values of 46 min (571) and 30 s (170) for the half-life of HA in whole brain. Neosynthesized [^{3}H]HA was localized in subcellular fractions containing nerve endings (571). However, the mode of administration of the tritiated precursor is unphysiological and results in a nonuniform labeling of various brain areas.

These drawbacks are avoided by intravenous administration of [^{3}H]His either in a pulse or at a constant rate (23, 660, 789). The mean half-life of HA in mouse brain was 46 min after constant rate infusion (660), a value similar to that found in rat brain (571), and 20 min after pulse injection (660). The values did not markedly differ between regions; however, there was a slightly shorter half-life in pons-medulla. The half-life of HA was much shorter in brain than in any peripheral organ (660).

2. Nonisotopic methods

Among the various experimental approaches that could theoretically be applied to the evaluation of HA turnover rate, only two have been extensively used; they consist of determining the rates of endogenous HA decline and *t*-MeHA elevation after inhibition of HDC and MAO, respectively.

After irreversible inactivation of HDC by α-FMH, HA levels decrease rapidly in mouse, guinea pig, and rat

brains (23, 235, 447, 507, 629). However, even a few hours after nearly total inactivation of HDC, the amine depletion is not complete, evidencing the presence of a resistant pool with a very slow turnover, the existence of which was already suggested in studies with less potent and reversible inhibitors (744, 745). The mean maximal HA depletion by α-FMH is ~50% but varies among CNS areas and animal species; in all species, no significant depletion occurred in the spinal cord (235, 507, 522, 629). The depletion of HA is rapid in a subcellular fraction containing nerve endings (23), indicating that HA turnover is rapid in neurons, but the cellular localization of the slowly turning over pool is still debated. In the brain of W/W^v mice, a mutant devoid of mast cells, HA levels are lower than in congenic normal mice and, in contrast to the latter, are almost completely depleted by α-FMH (447, 793, 833). However, this difference has not been confirmed in other studies (269, 335, 528).

The mean half-life of HA in the rapidly turning over pool is 30 min in rat, mouse, and guinea pig brains (23, 521, 522, 629). Whereas steady-state levels of HA vary markedly among regions, this is generally not the case for half-lives, but slightly higher values are found in the hypothalamus where HA perikarya are present.

After administration of pargyline, a MAO inhibitor, endogenous *t*-MeHA levels rise (238a, 337, 338, 521, 522, 629) at a rate that is likely to reflect that of HA release (571). From this rise, and with some assumptions, half-lives for HA can be derived that are generally in the same order as those obtained using synthesis inhibition or isotopic methods. Again the HA half-life is the longest in the hypothalamus, and the pargyline-induced rise in *t*-MeHA is negligible in spinal cord.

One potential limitation of these nonisotopic methods is that they require the administration of drugs in high dosages to completely block a metabolic pathway, and this could affect directly or indirectly the activity of histaminergic neurons. However, pargyline treatments, which are likely to affect several monoamines, do not generally modify HA steady-state levels (629) or turnover measured with [^{3}H]His (571).

3. *Drug-induced changes in turnover*

Histamine turnover in rat brain is reduced almost instantly on administration of barbiturates and a variety of hypnotics or anesthetics (37, 568, 571). Similar effects are observed with other "sedative" agents, such as the γ-aminobutyric acid (GABA)-mimetic drugs muscimol and benzodiazepines (506, 520), ethanol (356), and Δ^9-tetrahydrocannabinnol (519), the latter attributed to an inhibition of HA release. Other agents inhibiting HA release in vitro via stimulation of presynaptic histamine H_3, α_2-adrenergic, or muscarinic M_1 receptors reduce HA turnover in vivo (23, 270, 271). In contrast, reserpine, a drug interfering with the storage mechanisms of monoamines and releasing HA in vitro (747, 787), accelerates HA turnover (569). In vivo, blockade of H_3 receptors (23, 239, 519a) but not muscarinic or adrenergic receptors (270, 271) accelerates HA turnover, suggesting that only H_3 receptors are tonically activated in vivo.

Morphine (504), DAGO, a specific agonist at μ-opiate receptors (360), and [D-Ala^2,D-Leu^5]enkephalin, a nonspecific agonist at δ-opiate receptors, but not ethylketazocine, an agonist at κ-opiate receptors (359), enhance HA turnover. The psychotomimetic agent phencyclidine has a similar effect, which is blocked by naloxone, an opiate receptor antagonist (357, 359). After the various treatments modifying HA turnover, Saeki and co-workers (504, 519, 520) found that the steady-state level of *t*-MeHA was modified in an apparently unrelated manner, suggesting that this level does not constitute a reliable turnover index.

III. DISPOSITION OF HISTAMINERGIC NEURONAL PATHWAYS

In 1974, the decrease of HDC activity found in many rat brain areas after lesions of the lateral hypothalamic area was the first evidence for the existence of an ascending HA neuronal pathway with widespread projections to almost all regions of mammalian brain (232). Ten years later, the exact localization of HA perikarya in the posterior hypothalamus was established immunohistochemically using HA (552, 707) and HDC antibodies (802, 803). During the same period, HA neurons had been identified in *Aplysia* brain by biochemical analysis of microdissected tissues (813).

A. *Lesion Studies*

The rough disposition of HA pathways in rat brain, initially established by lesion studies, is now confirmed and obviously largely extended by immunohistochemical data. Hence these studies, which are reviewed in detail elsewhere (666), are rapidly summarized here.

Unilateral interruption of the medial forebrain bundle leads to ipsilateral decreases in HDC activity (by 50-55%) and HA levels (by 25–30%) in telencephalic and diencephalic areas rostral to the lesion (231, 232). The existence of ascending fibers emanating from the same bundle was also deduced from the effects of discrete lesions of the afferents to the hippocampal region (40) and amygdaloid nuclei (60). From the analysis of a series of discrete lesions in the mesodiencephalon a localization of corresponding perikarya in the mammillary region of the hypothalamus (and the neighboring mesencephalic reticular formation) was suggested (233). Finally, the existence of a descending HA pathway emanating from the posterior hypothalamus and projecting rather heavily to various brain stem nuclei was proposed (572).

Recently, unilateral lesions with ibotenic acid in the vicinity of the mammillary bodies (where HA perikarya are located) induced bilateral reductions in HA levels in the hypothalamus and frontal cortex but an ipsilateral

reduction in hippocampus. In contrast, HA levels increased markedly in the neurohypophysis (501).

B. Immunohistochemical Tools

The precise localization of HA neurons could be established with the advent of several reliable immunohistochemical tools. High-affinity polyclonal antibodies to a highly purified, although not homogeneous, preparation of HDC obtained from rat fetal liver (731, 803) have found the largest applications in several laboratories. These antibodies cross-react with dopa decarboxylase in guinea pig (but not rat) brain (16, 732) and apparently cannot be used for immunohistochemistry in species other than rats. The antibodies for HDC developed earlier (224, 758) were less specific. Despite its specificity, a monoclonal antibody to partially purified HDC from rat stomach has found only limited histochemical applications because of its rather low affinity (573-575).

Polyclonal antibodies to HA raised against the amine either simply mixed with serum albumin (531, 821) or conjugated to proteins using aldehydes (707, 708) or carbodiimide (550–552) have been also used as histochemical tools. Among these, the latter appear as the most reliable tools, especially when the detection sensitivity is enhanced by using carbodiimide as a tissue fixative (550). In contrast, other HA antibodies were found to detect numerous immunoreactive fibers or neurons in areas, such as the median eminence (707, 821), in which they seem absent or very scarce (351), an artifact presumably related to cross-reactivity with histidine-containing peptides, such as luteinizing hormone-releasing hormone (LHRH) (64).

C. Localization of Histaminergic Perikarya: Tuberomammillary Nucleus

Strikingly consistent results were obtained in various laboratories regarding the localization of histaminergic perikarya, whatever the immunohistochemical tool used, i.e., either the anti-HDC monoclonal (573, 574) or polyclonal antibodies (207, 350, 606, 803, 804, 829) as well as the anti-HA antibodies (550–552, 707, 708). Hence HA perikarya, mostly large cells, were found to be confined to the tuberal region of the posterior hypothalamus in an area where a group of Nissl-stained magnocellular neurons had been detected earlier and collectively named the tuberomammillary nucleus (TM) (165, 474; Fig. 1). Two groups of Nissl-stained magnocellular neurons were also detected later in the same area by Bleier et al. (80), who named them the caudal magnocellular nucleus (CMC) and the tuberal magnocellular nucleus (TMC). The same region is included in an "efficient area" defined by analyzing combined lesion data and proposed to contain HA perikarya projecting to the telencephalon (233). Other previous data are consistent with this localization: the neurotoxin kainate decreases HDC in this area, presumably by ablating HA perikarya, whereas colchicine has an opposite effect, presumably by blocking the enzyme axonal transport (233).

Whereas in previous studies HA perikarya were considered to belong to several distinct nuclei, generally named after Bleier et al. (80), Ericson et al. (207), in the most comprehensive anatomic study of this area performed at the light-microscopic level with the anti-HDC antibodies of Watanabe et al. (803), provided convincing evidence for the HA perikarya making up one continuous cell group. The latter, named the TM after Morgan (474) and a previous study of the same laboratory (411), was divided in several subgroups, generally in agreement with studies of other laboratories, although the nomenclature differs (for a correspondence see Fig. 1).

The medial tuberomammillary subgroup (TMM) is constituted of ~600 neurons situated on each side of the mammillary recess. It can itself be subdivided into *1*) a dorsal part (TMMd) corresponding to the TMC of Bleier et al. (80), i.e., an isolated group of cells extending over 1.5 mm along the tip of the third ventricule between the dorsal premammillary nucleus and the dorsomedial nucleus; and *2*) a ventral part (TMMv) close to the ventral surface of the brain just rostral to the medial mammillary body, occurring as a cluster of cells located dorsolaterally to the infundibular nucleus and the mammillary recess. This cluster is bridged (207, 827) by isolated immunoreactive cells with the ventral tuberomammillary subgroup (TMV).

The TMV, the largest one comprising ~1,500 mostly magnocellular neurons, is subdivided by the mammillary bodies into *1*) a rostral part (TMVr), corresponding to the CMC of Bleier et al. (80), situated just in front of the lateral mammillary nucleus; and *2*) a caudal part (TMVc), corresponding to the posterior caudal magnocellular nucleus of Bleier et al. (80), situated behind the lateral and medial mammillary bodies and the supramammillary nucleus.

The diffuse part of the tuberomammillary nucleus (TMdiff) is constituted by a small number (~100) of HDC-immunoreactive cells scattered within or between various hypothalamic nuclei in the lateral hypothalamic area, the posterior hypothalamic area, the perifornical area, and the supramammillary and dorsomedial nuclei. Most of them were detected in previous immunohistochemical studies (573, 574, 707, 708, 803, 804), but they were only recently considered (207, 411) as belonging to a single subset.

Histamine perikarya are immunodetectable in rat hypothalamus during late fetal stages (35, 605) and can be cultivated for several days in vitro (550).

Although most anatomic studies were performed in rats, the TM was first identified in Nissl-stained sections of the dog hypothalamus (474) and seems present in all mammals, becoming more differentiated in primates and most extensive in humans (165, 411). With the use of antibodies against HA, perikarya were also localized in the mammillary region of guinea pigs: they seem more numerous than in rats, being also found between the medial and lateral mammillary nuclei as well as between the premammillary nuclei (7). In cat hypothalamus, magnocellular neurons becoming immunoreactive to 5-HT antibodies after treatments with 5-hydroxytryptophan (5-

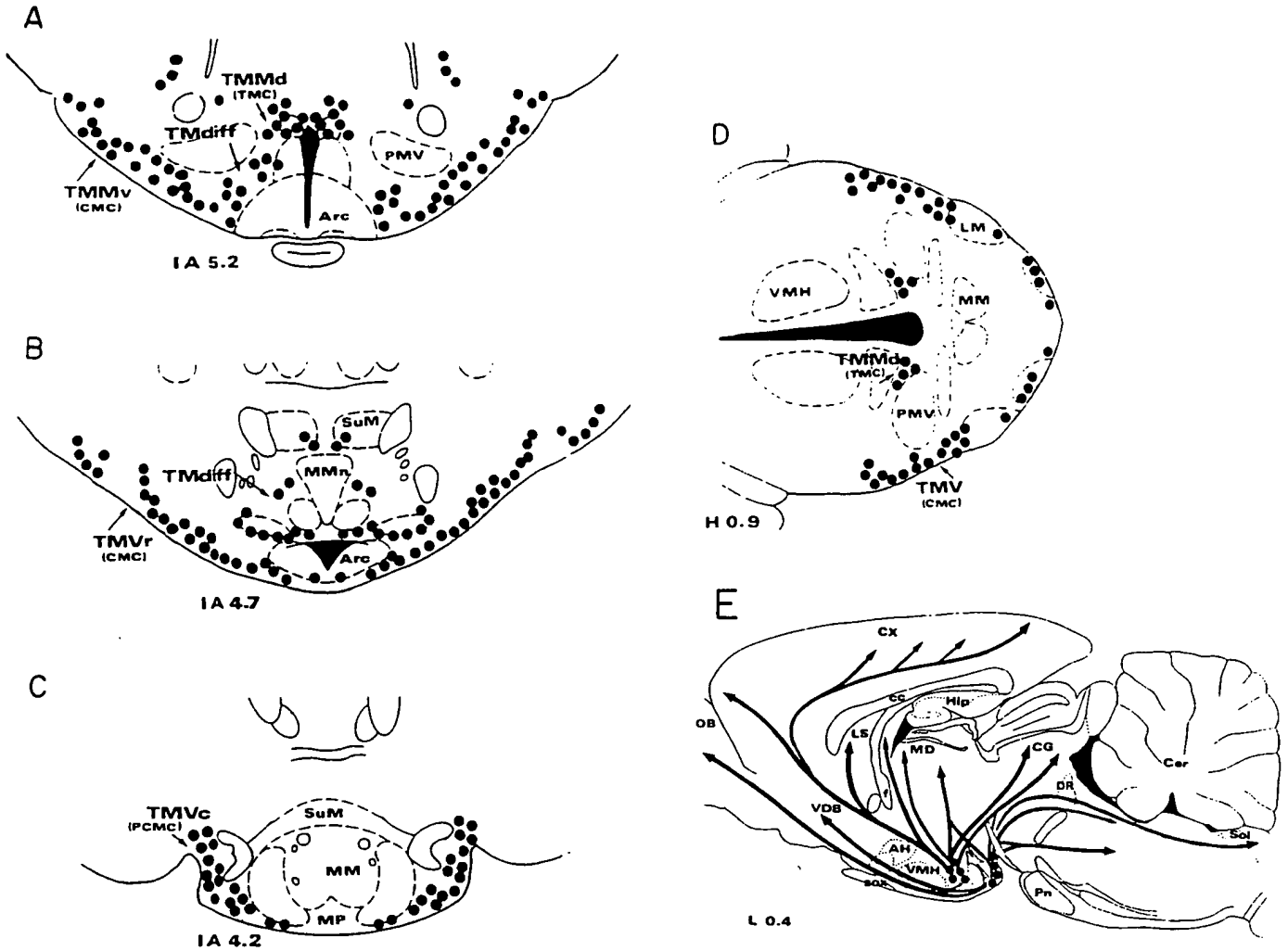

FIG. 1. Localization of histaminergic perikarya (closed circles) in tuberomammillary nucleus and disposition of main histaminergic pathways (arrows) in rat brain. Sections represented according to atlas of Paxinos and Watson (553a). The subgroups of perikarya in tuberomammillary nucleus are designated according to Morgan (474), Diepen (165), and Köhler et al. (411), and corresponding nomenclature according to Bleier et al. (80) is indicated within parentheses. *A*, *B*, and *C*: frontal sections at indicated levels of caudal hypothalamus. *D*: horizontal section through caudal hypothalamus. *E*: sagittal section of brain. Abbreviations of perikarya subgroups: CMC, caudal magnocellular nucleus; PCMC, posterior caudal magnocellular nucleus; TMC, tuberal magnocellular nucleus; TMdiff, tuberomammillary nucleus diffuse part; TMMd, medial tuberomammillary subgroup dorsal part; TMMv, medial tuberomammillary subgroup ventral part; TMV, ventral tuberomammillary subgroup; TMVc, ventral tuberomammillary subgroup caudal part; TMVr, ventral tuberomammillary subgroup rostral part. Other abbreviations: AH, anterior hypothalamic area; Arc, arcuate nucleus; cc, corpus callosum; Cer, cerebellum; CG, central gray; CX, cerebral cortex; DR, dorsal raphe nucleus; f, fornix; Hip, hippocampus; LM, lateral mammillary nucleus; LS, lateral septum; MD, mediodorsal thalamus; MM, medial mammillary nucleus medial part; MMn, medial mammillary nucleus median part; MP, medial mammillary nucleus posterior part; OB, olfactory bulb; PMV, premammillary nucleus ventral part; Pn, pontine nuclei; Sol, nucleus of solitary tract; Sox, supraoptic decussation; SuM, supramammillary nucleus; VDB, nucleus of vertical limb of diagonal band; VMH, ventromedial hypothalamic nucleus.

HTP) seem to correspond to HA neurons and extend from the suprachiasmatic nucleus to the caudal end of the hypothalamus (632, 636, 637). Histamine-immunoreactive perikarya were also detected in the human mammillary region (549, 549a). In all these species the HA magnocellular neurons seem less aggregated than in rats but otherwise display very similar features.

In one study immunoreactive HDC (irHDC) horizontal cells were detected in the guinea pig retina (17), but this could not be confirmed using an anti-HA antibody (7).

D. Morphology of Histaminergic Neurons

The HA neurons of the TM display a series of characteristic features, as revealed in the various immunohistochemical studies at the light-microscopic level (see sect. III*C*) or electron-microscopic level (296, 825, 829).

Most of them are large (25–35 μm) neurons, particularly in the TMV (since the TMM contains a large number of medium-sized cells), with a round unindented nucleus, a well-developed Golgi apparatus, and a relatively large

nount of karyoplasm. Very similar ultrastructural aracteristics are found in noradrenergic or serotoninerc perikarya as well as in the cholinergic perikarya of e basal nucleus of Meynert.

Another characteristic feature at TM cells is their ndritic processes comprising two to three thick priary dendrites dividing into long (100-150 μm) secondary ndrites, with few spines and axodendritic synaptic ntacts. The dendritic trees of adjacent neurons overlap ch other. In one study it was observed that the denites narrowed down into varicose fibers filled with all vesicles similar to synaptic vesicles, and it was hythetized that exocytosis might occur at this level (445), t this was not confirmed in another study in which the ricose fibers emerging from the emission cones of denites were identified as axons (825).

Another very characteristic feature is the close in-action of dendrites with glial elements in the mamllary recess and the ventral surface of the brain in a y suggesting that the dendrites penetrate into the endyma and come into close contact with the CSF. The act functional significance of this peculiar organizan is still obscure, but it has been proposed that HA urons might either release their secretions into the F (445) or respond to CSF-borne substances (606).

Finally, in one ultrastructural study, both myelind and unmyelinated irHDC varicose axons, making v typical synaptic contacts, were observed in the TMV 4). This was interpreted as evidence for a mainly nonaptic release of HA, similar to that proposed for other noamines (57), but the relationships of these elements h the varicose dendrites of Maeda et al. (445) remains be clarified.

Other Putative Transmitters in Histaminergic Neurons

The HA neurons in TM are characterized by the unal presence of a large variety of markers for other rotransmitter systems, some of which evidenced be e or independently from the demonstration of HA itf.

Glutamic acid decarboxylase, the GABA-synthesizenzyme, was first evidenced in TM neurons projecting he cerebral cortex by Vincent et al. (791), and its coloization with the vast majority of irHDC neurons (679, , 825) was shown soon later.

In a parallel manner, Nagy et al. (478) evidenced in neurons the presence of adenosine deaminase, a cytosmic enzyme that might be responsible for the inactiion of the putative neurotransmitter adenosine. Pres:e of this marker in the vast majority of irHDC ikarya was demonstrated soon after (553, 679,). Tuberomammillary nucleus neurons also express ding sites for a putative ligand of adenosine uptake s (477).

A majority of irHDC neurons also stain with antilies against galanin, a 29-amino acid neuropeptide), 466, 700). Interestingly galanin is also colocalized with other amines, i.e., catecholamine, 5-HT, and acetylcholine in other cerebral neurons (466).

Also a majority of TM neurons stain with antibodies raised against the opioid heptapeptide (Met^5)enkephalyl-Arg^6-Phe^7, suggesting that they express the proenkephalin A gene (411, 822).

Staining of at least a fraction of TM neurons with antibodies against two other neuropeptides, i.e., substance P (411) or thyrotropin-releasing hormone (TRH) (688) was also reported.

Another characteristic feature of a rather large subpopulation of TM neurons is the presence of high MAO B activity, possibly related to deamination of *t*-MeHA (392, 393, 444, 445, 700, 730). This subpopulation, representing ~60% of neurons expressing both HDC and adenosine deaminase, could be the same as that expressing enkephalins (730).

Finally, a fraction of TM neurons have the capacity to uptake and decarboxylate 5-HTP into 5-HT (which is then detected immunohistochemically) but not to synthesize 5-HT after tryptophan administration (445, 632, 637, 700). This suggests that some TM neurons express not only HDC, for which 5-HTP is not a substrate, but also the aromatic amino acid decarboxylase (EC 4.1.1.28) and thereby resemble the peripheral APUD cells (554).

The fact that such a high number of neurotransmitter markers are apparently expressed by the same TM neurons raises several comments: *1*) these colocalizations have been established by purely histochemical approaches and require confirmation by other approaches; *2*) the biological significance of some markers, e.g., adenosine deaminase or 5-HTP uptake and decarboxylation activities, is not fully clarified; and *3*) the delineation of neuronal subpopulations within the TM, taking into account which among the eight markers listed above are coexpressed by each cell, has only started (410, 679, 700). Finally, and most importantly, establishing the functional significance of the colocalization of such a high number of messengers in a single population of neurons still remains an important and exciting challenge.

F. Pathways and Projection Fields of Histaminergic Neurons

Although some fibers were detected in the early immunohistochemical studies (552, 573, 574, 707, 708, 731, 805), it is only recently that technical improvements have started to reveal the extensive networks of irHDC (350) and irHA (7, 551) fiber systems in brain that were already suggested by lesions (232). This information has been complemented by that provided by retrograde tracing studies, sometimes performed before the realization that HA perikarya were located in the TM.

Histamine neurons constitute long and highly divergent systems projecting in a diffuse manner to many cerebral areas (232), with immunoreactive varicose or nonvaricose fibers being detected in almost all regions of frog (7a), rat (350, 551), guinea pig (7), tree shrew (6a), or human brain (549, 549a). Despite initial suggestions that

the various TM subgroups might have distinct projection fields (708, 709), more recent retrograde tracing studies have established that TM neurons are not organized in a highly topographic way, since individual neurons give rise to projections to widely divergent parts of the forebrain, cerebellum, and mesencephalon (207, 411).

Panula et al. (551) distinguished two ascending pathways in rat brain. The ventral pathway runs at the basal surface of the brain toward the horizontal limb of the nucleus of the diagonal band and then to the medial septal nucleus or the olfactory tubercle and bulb, with a large fraction of the fibers crossing the midline in the retrochiasmatic area. The dorsal pathway runs along the lateral side of the third ventricle and contributes to fibers ending in thalamus and rostral forebrain structures (Fig. 1).

Moderately dense HA fibers are quite evenly distributed in all areas and layers of the cerebral cortex (7, 350, 551, 707, 708, 735, 803). In human cerebral cortex they appear to be most abundant in the external layers where they run in parallel to the surface (549, 549a). Deafferentation studies have established that they are extrinsic (41, 232, 735), and retrograde tracing studies showed that they emanate from the TM (207, 411, 478, 632, 708, 709, 784, 791), ascending through the medial forebrain bundle itself (232), which contains numerous fibers in rats (350, 551) or in guinea pigs an area between this bundle and the optic tract (7).

From studies combining lesion and biochemical (231–233) or immunohistochemical (735) analysis of HDC, it appears that the cerebral cortex receives predominantly ipsilateral projections of HA fibers. However, a minor participation of a contralateral projection, representing only about one-fifth to one-third of the total, was shown by retrograde tracing (411, 478, 735, 791) and antidromic activation studies (604). Midline crossing of these fibers occurs at the level of the retrochiasmatic area, the optic chiasma, and in the supramammillary region (350, 551) and was overlooked in combined unilateral lesion and biochemical studies (231, 232), presumably because of its limited extent and inherent individual variation among control (nonlesioned) animals. The extent to which crossing of fibers originating from the TM occurs seems to vary according to the innervated brain area, but there is, in most cases, a majority of ipsilateral fibers (207, 411).

In a variety of other telencephalic areas, immunohistochemical studies, sometimes combined with retrograde tracing, have shown long ascending connections between perikarya in the TM and the olfactory bulb (mainly in the external plexiform layer), hippocampus (mainly in dentate gyrus and subiculum), nucleus accumbens, globus pallidus, and amygdala (7, 207, 350, 550, 551, 708, 709, 828). In the caudate-putamen, fibers are surprisingly scarce when detected with anti-HA antibodies (551) but are considerably more numerous when detected with anti-HDC antibodies (350, 803). This general organization of HA pathways is in agreement with previous deafferentation or focal lesion-induced changes in HDC in some of these areas, i.e., the amygdaloid complex in which a very rich HA innervation is present (60) or the hippocampus (40). These studies suggested that, in rats, HA fibers reach the amygdaloid complex via the ansa peduncularis and the hippocampus via a dorsal route comprised of the fimbria, fornix superior, and cingulum; in agreement immunoreactive fibers seem abundant in rat fimbria (350, 551) but very scarce in the guinea pig fimbria and fornix (7).

In the diencephalon, the most densely innervated part of the brain, various nuclei of the anterior and medial hypothalamus, e.g., the suprachiasmatic, supraoptic, paraventricular, arcuate or ventromedial nuclei, as well as of the thalamus, e.g., paratenial and paraventricular nuclei, contain numerous HA fibers (7, 350, 551, 552, 573, 708, 803). Hypothalamic nuclei contain high levels of HA and HDC activity (98, 567), with the latter being decreased after more caudal hypothalamic lesions (232, 233). In the median eminence only, scarce immunoreactive fibers are detected, mainly in the internal layer (7, 350, 351, 551), confirming that the high HA level in this area mainly corresponds to mast cells (567). In the hypophysis, fibers in moderate density occur in the posterior lobe but seem absent in the other lobes (7, 350). Bilateral projections from the TM to widespread regions of the hypothalamus were evidenced by retrograde tracing (701).

Finally, a long descending HA system, also arising from the TM and projecting to various brain stem structures and the spinal cord, was evidenced by lesion (233, 572), immunohistochemical (7, 350, 352, 551, 708, 795), and retrograde tracing studies (207, 632). Interestingly, double tracing studies have suggested that a single TM neuron might send both ascending and descending projections (207), but this is not consistent with other observations (632, 733). Fibers travel caudally in association with the medial longitudinal fasciculus in rats (795) and with the dorsal fasciculus and medial periventricular gray in guinea pigs (7). The most densely innervated structures in the brain stem are the mesencephalic nucleus of the trigeminal nerve, the central gray, the colliculi, and the nucleus of the solitary tract, whereas other cranial nerve nuclei seem less abundantly innervated (7, 350, 352, 572). With the use of antibodies against HA, moderate to dense networks were observed in the substantia nigra and raphe nuclei of guinea pigs (7) as well as of rats (551) in agreement with biochemical (572) but not immunohistochemical data obtained with antibodies against HDC in rats (350). A direct projection of the TM to the locus coeruleus was shown by retrograde tracing (632, 724).

In the cerebellum, irHA fibers are sparsely distributed in all cortical layers and nuclei (7).

In the dorsal horn of the spinal cord (mainly the cervical part), fibers originating from the TM are moderately abundant and some cross the midline (7, 795).

Immunoreactive HA fibers are observed in the wall of intracerebral blood vessels in rats (710) but not in guinea pigs (7).

G. Ontogeny of Histaminergic Neurons

The ontogeny of irHA (35) and irHDC neurons (60) was recently studied in rat brain. The first irHA perikarya were seen on embryonic *day 13* in the border

etencephalon and mesencephalon and on *day 15* in the ntral mesencephalon, metencephalon, and myelenphalon. From these scattered cells a transient ascendg and descending fiber system starts to develop on emyonic *day 15* but has completely disappeared by *day 20*. contrast, in the basolateral hypothalamus, irHA (35) id irHDC cells (605) were first detected on embryonic y *16* when they had stopped their mitotic division. The fferentiation of immunoreactive neurons in the various bgroups of the caudolateral part of the tuberomamillary nucleus seems largely achieved by embryonic *day* , whereas the appearance of the dorsal subgroup in the edial part of the nucleus only occurs during the last enatal days. Adenosine deaminase is first detected in beromammillary neurons on embryonic *day 18* (679).

The development of most irHA fibers takes place ring the first two postnatal weeks (35), which coincides ith the developmental pattern of HDC activity taken as selective marker of HA neuronal pool (457).

In contrast, irHA mast cells are most numerous on stnatal *day 4* (when they are mostly located in the hipcampal area), and after that, their number gradually creases (35), a pattern that explains changes in HA vels, as proposed (457).

Afferents to Histaminergic Neurons

Little is known about the sources and biochemical entity of neuronal afferents to HA neurons, namely ecause their location as a thin sheet of perikarya at the asis of the brain severely restricts the application of trograde tracing substances. Anterograde tracing studs had shown that substantial projections to the poster hypothalamus arise from the olfactory tubercle, suculum, septum, medial preoptic area, and dorsal tegentum (725), but their actual projections to HA erikarya were not established. Recently, however, Wourlood and colleagues have started to apply to this prob m a double-label immunocytochemical method (826), sing antibodies against HDC and *Phaseolus vulgaris* ucoagglutinin, an anterograde tracer. In this way prections from the infralimbic division of the prefrontal rtex (830), all nuclei of the septum diagonal band comlex (except the medial and lateral parts of the horizonal limb of the diagonal band) (828), and the medial reoptic region of the hypothalamus (827) were evienced at the light-microscopic level. The pathways for ie afferent fibers could be traced and varicosities on iese fibers observed in proximity to irHDC cell bodies nd dendrites, suggesting the possible occurrence of synptic contacts. Because the areas of origin of these afferits receive, in most cases, heavy histaminergic inputs, a eciprocal control with TM neurons was postulated. lowever, blockade of postsynaptic H_1 and H_2 receprs does not affect HA turnover in cerebral corex (239).

Afferent serotoninergic and noradrenergic fibers ere also described in the TM (207). Although the exisence of direct connections remains to be established, the presence of α_2-adrenergic receptors on HA cells (271) may reflect a noradrenergic input.

IV. NONNEURONAL STORES OF HISTAMINE IN BRAIN

Outside the brain mast cells represent a major site of HA storage, and their exposure to antigen (via interaction with immunoglobulin E) or to histamine releasers (e.g., compound 48/80, a basic polymer) leads to extrusion of HA, heparin, and proteases contained in their granules. In peripheral tissues, mast cells are often found in connective tissue, in proximity to vascular elements, and for a long time they were thought to be absent from brain, which contains very little connective tissue. In the early 1970s they were progressively detected by their metachromatic staining by dyes such as toluidine blue (111, 189, 342, 390, 419, 524, 559), and their significant contribution in cerebral HA levels was proposed (457, 654). However, although the existence of this nonneuronal pool of HA is generally admitted, its relative quantitative importance is still debated (328, 584), the function of brain mast cells essentially is unknown (747a), and the existence of additional nonneuronal pools is postulated.

A. Mast Cells in Brain

In peripheral tissues, mast cells are essentially defined by their morphological properties, e.g., metachromasia or alcian blue staining of their very large granules. However, they constitute a heterogeneous population with two main subtypes, the connective tissue and the less typical mucosal mast cell (206). The mast cell subtypes are distinguished by their morphology, staining properties, HA content, composition (e.g., intragranular proteases), and sensitivity to HA releasers (206).

In brain, mast cells, detected by their metachromasia (111, 189, 190, 332, 419, 524, 559), the HA, and in rats 5-HT histofluorescence (199), HA immunoreactivity (7, 550, 552, 707), or their ultrastructure (166, 343), are present in a large number of species (190), including humans (199, 202). Mast cell numbers and localization in brain are characterized by striking variations according to regions, species, individuals, periods of the year, age, sex, side of the brain, and even handling of the animal (for review see Refs. 328, 332, 559, 747a), which may be real or related to the limited reliability of the staining procedure. Nevertheless, from most studies, mast cells seem generally abundant in leptomeninges, pituitary, pineal gland, area postrema, or median eminence but also occur in the brain parenchyma itself, mainly the grey matter, where, similar to their counterparts in peripheral tissues, they are distributed along vessels. The reported number of mast cells in rat brain varies between a few hundreds to >10,000, among which a fraction as large as 80% is found in the thalamus, particularly its lateral nuclei (189, 190, 251, 332, 559). However, in other species the distribution might differ with, for instance, a high density in the cerebral cortex of cats (343) or chimpanzees (190).

The fraction of rat brain HA contained in mast cells has been estimated to be ~50% from lesion and subcellular fractionation studies (231, 457, 654) as well as from mast cell counts combined with HA assays (332); however, others suggested this fraction to be negligible (584). Studies of mutant W/W^v mice deficient in mast cells did not clarify this issue, because their cerebral HA levels were found to be decreased compared with corresponding controls in two laboratories (447, 793, 833) but not in two other laboratories (335, 528).

A large fraction of HA might be held in mast cells in the neonatal rat brain (457) in which they seem more numerous than in the adult (7, 190, 213). This feature would explain *1*) the relatively high HA level just after birth (555, 619, 669, 749, 836); *2*) the distinct properties of HA-storing granules in neonatal brain, which sediment like those of mast cells (457, 565, 836); *3*) the low turnover of HA, which resembles more that of the amine in typical mast cells than in the adult brain (457); and *4*) the clear in vitro releasing effect of compound 48/80 (457), whereas in the adult brain this effect only occurs with slices of regions, such as the median eminence, where mast cells are abundant (567). Mast cells could proliferate in the neonatal brain under the action of nerve growth factor (11).

Ultrastructural studies (166, 343, 344, 346–348, 713) have all confirmed the close association of brain mast cells with blood vessels and allowed Ibrahim (343) to distinguish two types among these cells. Type I cells are those with morphological properties, including metachromasia, identical to those of typical, i.e., connective tissue, mast cells, which would be those reported by most investigators. Type II mast cells were later called neurolipomastocytes because of the high lipid content of their large granules, which are stained by toluidine blue but are not metachromatic or alcianophilic, although they otherwise display close ultrastructural similarity with typical mast cells. Neurolipomastocytes, which seem more numerous than typical mast cells, enter the brain with pial blood vessels and are always associated with arterioles or venules at the site of vessel branching where autonomic innervation of pial vessels is the densest (166, 713). In these cells, the presence of HA has not been directly demonstrated, but they degranulate under the action of compound 48/80 (166, 347, 348). As mast cells in peripheral tissues (496, 702, 820), neurolipomastocytes in pial arteries of rats and rabbits are autofluorescent cells often situated in apposition with variosities from nerves of sympathetic and other origin, suggesting that their function (secretion?) might be neurally controlled (166, 747a).

Although the function of cerebral mast cells is not established, the localization, innervation, and content of these cells suggest their participation in the regulation of cerebral blood flow and inflammatory reactions (747a).

B. Other Nonneuronal Stores

Histofluorescence studies have suggested the association of HA with cerebral vascular elements (204, 332). In agreement, a relatively high HA level is found in isolated cerebral microvessels in which it is associated with a low HDC content, suggesting that, therein, the amine turns over slowly (362, 381, 382, 616). Because this fraction did not contain metachromatic cells, it was assumed that HA was held in endothelial cells (616), which may contain a still poorly characterized pool of "nascent HA," the formation of which is induced by hormones or various inflammatory agents (370, 646).

The unexpected rise in cerebral HA level that accompanies several large lesions (168, 418) may correspond to this poorly clarified process.

V. HISTAMINE RECEPTORS IN BRAIN

The idea that the various actions of HA on peripheral tissues were mediated by more than one receptor subtype emerged progressively. It was first realized that the antihistamines (now termed H_1-receptor antagonists), the first of which was developed in France in 1937 (87), did not block uniformly all actions of HA, leaving for instance gastric acid secretion unaffected. In 1966, Ash and Schild (31), demonstrating that these various actions could also be differentiated to a certain extent by agonists, clearly postulated the existence of the second (H_2) receptor subtype that was "proven" in 1972 with the development by Black et al. of burimamide (77), the first "selective" antagonist (in the sense that it has no significant affinity for H_1 receptors). Three years later, both H_1 and H_2 receptors were shown to be present in mammalian brain where they control cAMP formation (56, 618) and the firing rate of cortical neurons (279). In 1976, the association of H_2 receptors with adenylate cyclase (298) and that of H_1 receptors with the phosphatidylinositol cycle (361) were shown. Finally, in 1983, Arrang et al. (25) proposed the existence in brain of the H_3 receptor (for which, ironically, burimamide was found to be the most potent antagonist available at this time), which was "proven" in 1987 with the design of fully selective agonist and antagonist compounds (23).

The experimental definition of the three HA receptor subtypes relies on the use of selective agonists and antagonists that are now available, except, however, a highly selective H_1-receptor agonist. Various aspects of the field of cerebral HA receptors were recently reviewed (105, 137a, 256, 304, 305, 658, 659).

A. Histamine H_1 Receptors

1. Molecular properties

The antagonist [^{3}H]mepyramine was introduced in 1977 as the first selective radioligand for H_1 receptors (313) and still displays advantages over other tritiated ligands, i.e., the antidepressants [^{3}H]doxepin (3, 375, 739, 752, 757) or [^{3}H]mianserin (558) as well as (+)-*N*-methyl-4-[^{3}H]methyldiphenhydramine, a quaternary derivative of diphenhydramine (759, 760), that appear less

lective. A mepyramine derivative [^{125}I]iodobolpyramine (78, 414) is also a highly selective probe for the H_1 ceptor, which it labels reversibly with high affinity nd provides a 50-fold increase in detection sensitivity ver [^{3}H]mepyramine (414, 622). Tritiated mepyramine as proposed (589, 591) for H_1-receptor labeling in rains of living mice and as a means of predicting the otential sedating ability of H_1 antihistamines and a ariety of antipsychotics and antidepressants (589). his test is currently applied to assess the brain penetation ability of potentially nonsedating H_1-receptor ntagonists (6, 109, 429, 620).

The pharmacology of H_1 receptors differs among nimal species, since some antagonists, e.g., mepyramine, display significantly lower affinities in rat (or ouse and rabbit) brains than in guinea pig or human rains (3, 73, 117, 120, 121, 306, 311, 377, 414, 755, 797). his rather unusual species difference is confirmed hen a functional response, i.e., HA-induced glycogenolsis, is considered (590). The binding of HA to H_1 recepors is inhibited, although to a limited extent, by guaylnucleotides (119, 483, 622), and the effect is lost on olubilization (752). This suggests that the H_1-receptor regulated by a G protein that is presumably distinct om the N_i protein, because pertussis toxin is inactive HA binding (483). Sodium ions also decrease HA affnity at H_1 receptors, but the functional significance of is effect is unclear (119). Both thiol-alkylating (240, 4) and the disulfide bond-reducing agents (173–175) abilize a fraction of the H_1 receptors in a high-affinity ate for HA and full agonists, presumably by modifyg a critical cysteine residue(s) located outside the HA nding domain (834).

The H_1 receptor can be solubilized using digitonin 40, 243, 752) or other detergents (223, 799). In the solulized state, it is retained on a wheat-germ agglutinin olumn, indicating its glycoprotein nature (240), and e molecular sizes of the receptor-detergent complexes etermined by gel filtration are 430,000 (752) and 0,000 (223).

The molecular size of the H_1 receptor from various ssues, as determined by target size analysis, ranged om 100,000 to 160,000 (424, 798). A [^{3}H]mepyramine nding site abundant in rat liver membranes has been rtially purified, but it does not pharmacologically rrespond to the H_1 receptor (223).

A mepyramine derivative [^{125}I]iodoazidophenpyraine is an extremely potent H_1-receptor antagonist (K_D = 10 pM) that, after irradiation, selectively and irrersibly labels a 56-kDa subunit, presumably representg the ligand binding domain of the receptor in guinea g brain (622, 624) as well as in a variety of other tissues 24). In contrast, the H_1-receptor subunit labeled in art has a larger apparent mass, suggesting that rious tissues may express distinct isoforms of this reptor (622).

Distribution in central nervous system

The number of cerebral H_1 receptors varies among ecies but the mean density, i.e., ~100 fmol/mg membrane protein, is in the same range as that of receptors of other neurotransmitters, which are generally more abundant in brain than HA (121, 307, 311, 375, 414, 592, 622, 623). Not only the density of H_1 receptors but also their regional distribution markedly varies between animal species, with, for instance, the cerebellum being the densest area in the guinea pig and the least dense in rat or human brain (121, 375). In human brains, the highest [^{3}H]mepyramine binding is found in the neocortex and various limbic structures (121, 375). These species differences reflect only partly those in histaminergic innervation that appear much less marked (7, 551).

Several cell types might express the H_1 receptor in brain. Kainate lesions decrease H_1 receptors in guinea pig cerebellum, suggesting their association with neuronal membranes (544), but they do not affect them in several areas of rat brain, which may reflect a predominantly nonneuronal localization (122). Although H_1 receptors are present on cerebral microvessels, they represent only a small fraction of the brain's total content (557). The H_1 receptors have also been located on human astrocytoma cells (483).

In contrast, the predominant localization of H_1 receptors to grey matter areas and their highly heterogeneous and sometimes laminar distribution, as established autoradiographically at the light-microscopic level using [^{3}H]mepyramine (543–545) or [^{125}I]iodobolpyramine (86, 414), are consistent with a major neuronal localization.

A detailed atlas of H_1 receptors in guinea pig brain has been established using [^{125}I]iodobolpyramine, which, like other ^{125}I probes, displays distinct technical advantages over ^{3}H probes in autoradiographic studies (86). In the cerebral cortex, H_1 receptors are present in all areas and layers, with a higher density in lamina IV. In the hippocampal formation, H_1 receptors display a laminated pattern of distribution and are the most abundant in the dentate gyrus (hilus and molecular layer) and in several areas of the subiculum and commissural complex. In the amygdaloid complex, the highest densities are found in the medial group of nuclei. In the basal forebrain, the striatum is faintly labeled, whereas the nucleus accumbens, islands of Calleja, and most septal nuclei are highly labeled. In the thalamus, H_1 receptors are present in high density, particularly in the anterior, median, and lateral groups of nuclei. In the hypothalamus the labeling is highly heterogeneous with high densities in, for example, medial preoptic area, dorsomedial, ventromedial, and most posterior nuclei, including the tuberomammillary complex in which histamine perikarya and short axons are present. In the cerebellum the molecular layer is densely labeled (86, 414, 543), but H_1 receptors are also detectable in the granular layer and inner nuclei (86, 414). In normal mice, the pattern is similar, and changes occurring in mutant strains are compatible with a predominant localization to Purkinje cells (621). In guinea pig mesencephalon and lower brain stem, H_1 receptors are particularly abundant in the nuclei of origin of most cranial nerves, in areas containing the perikarya of the catecholamine and 5-HT systems, and in various areas associated with vegetative reflexes,

e.g., the area postrema. The H_1 receptors are abundant in cochlear nucleus in rats (543) and mice (221). In the spinal cord, the highest density is found in the external layers of the dorsal horn in guinea pigs (86) as well as in monkeys (502, 503).

There are several discrepancies between the distributions of HA terminals (7) and H_1 receptors in guinea pig brain (86) that recall similar "mismatches" in other neurotransmitter systems (85, 300, 422). The increase in [^{3}H]mepyramine binding in the developing rat brain parallels that of HDC, a presynaptic marker of HA neurons (134, 720, 756), as well as the HA-induced phosphoinositide hydrolysis (132). Whereas no change in cerebral H_1-receptor density occurs after long-term inhibition of HA synthesis in adult mice (T. T. Quach and J. C. Schwartz, unpublished observations) in newborn rats, their density increases after chronic treatments with a H_1-receptor antagonist (720) and decreases after treatment with an antithyroid agent (134).

3. *Inositol phospholipid hydrolysis*

The observation that responses mediated by H_1 receptors are Ca^{2+} dependent led to the proposition that this receptor subtype was linked to calcium mobilization (656). An early event associated with the stimulation of several calcium-mobilizing receptors is the hydrolysis of inositol phospholipids in the plasma membrane that occurs via activation of the enzyme phospholipase C and releases two types of intracellular messengers: inositol phosphates (inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate), which mobilize Ca^{2+}, and 1,2-diacylglycerol, which activates the intracellular protein kinase C (66, 468, 508). Even before general acceptance of the phosphatidylinositol cycle as a major transduction mechanism, it was shown that intracisternal HA accelerates the incorporation of inorganic ^{32}P into inositol phospholipids (220) via H_1-receptor stimulation (719). Meanwhile, mepyramine-sensitive stimulation of phosphatidylinositol turnover by HA was shown to occur in the guinea pig ileum (361). In a more direct study of the initial steps of the cycle, H_1-receptor stimulation was shown to trigger the rapid accumulation of [^{3}H]inositol 1-phosphate in brain slices incubated in the presence of [^{3}H]inositol and Li^+, an inhibitor of inositol 1-phosphatases (65, 97, 115, 116, 131, 158, 159, 173, 386, 831). Other inositol phosphates, i.e., inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate, are also rapidly accumulated under similar conditions (115, 173, 174). The amplitude of the phosphoinositide response is correlated with the H_1-receptor density in regions of guinea pig brain (115, 158, 159) or during brain development in rats (720). Interestingly, in guinea pig cerebral cortex, the half-maximal concentrations of HA to trigger the phosphoinositide response and the inhibition of [^{3}H]mepyramine are similar (115, 158, 159), and both parameters are similarly reduced by partial irreversible inactivation of H_1 receptors (131). This indicates that the response occurs without any significant receptor reserve, an observation consistent with this response playing a primary role in signal transduction. However, this does not apply to the response in cerebellum on which the effects of partial agonists also suggest a different coupling efficiency (115, 173). The fact that the response to HA is markedly reduced by calcium chelators (115, 386) does not rule out that it is responsible for Ca^{2+} translocation, since this may only reflect the requirement of a minimal Ca^{2+} concentration for phospholipase C activity (353). In cultured vascular smooth muscle cells, H_1-receptor stimulation elicits a transient increase in intracellular Ca^{2+}, which is presumably due to its intracellular mobilization, since it persists in the absence of extracellular Ca^{2+} (459). In contrast with a previous report (695a), H_1-receptor stimulation was also found to increase intracellular Ca^{2+} in neuroblastoma cells, but the response was attributed mainly to the increased entry of extracellular Ca^{2+} and led to hyperpolarization via activation of K^+ channels (517, 518).

Adenosine, despite having no direct effect alone, potentiates the H_1 receptor-mediated hydrolysis of inositol phosphoinositides elicited by HA in guinea pig cerebral cortical slices (308, 309, 321). In contrast, adenosine (385) and GABA (146), which coexist in HA neurons, both inhibit the HA-induced response in slices of mouse and rat cerebral cortex, respectively. Excitatory amino acids also inhibit the HA-induced response in hippocampal slices (54).

4. *Potentiation of adenosine 3',5'-cyclic monophosphate accumulation*

Histamine is among the most powerful stimulants of cAMP accumulation in brain slices (153, 371), and this is attributable to the participation of both H_1 and H_2 receptors in this response (56, 169, 618). Whereas only H_2 receptors are directly linked to the cyclase and the increase in cAMP formation elicited by their activation can be observed in cell-free preparations (298), the effect mediated by H_1 receptors is an indirect one, consists of a large amplification of the cAMP response mediated by H_2 receptors (from a 2- to 3-fold to up to a 15-fold increase), and can be evidenced only in intact cell preparations, such as slices of guinea pig hippocampus conjointly stimulated by H_2-receptor agonists (539). Both H_2 and H_1 receptors mediate the cAMP response to HA in low and high concentrations, respectively. A similar process was shown to operate in slices of rabbit cerebral cortex (8) as well as in vesicular entities (microsacs) of guinea pig cerebral cortex (154, 155, 587).

Stimulation by the H_1 receptor may amplify the cAMP response via one of the two signals known to be generated by the phosphatidylinositol cycle. However, although the H_1 receptor-mediated response is not modified through activation of protein kinase C by phorbol esters (238a), it is significantly reduced in the absence of Ca^{2+} (8, 238b, 661, 666), suggesting that the inositol phosphate branch of the cycle and the Ca^{2+} mobilization that it triggers play a major role. Intracellular Ca^{2+} may activate the adenylate cyclase via interaction with

odulin-calmodulin binding protein complex in the catytic subunit of the enzyme.

In a parallel manner, stimulation of H_1 receptors nplifies the cAMP response generated in intact cell eparations by other direct activators of the cyclase, g., adenosine (160, 169, 172, 320, 321), catecholamines ting via α-adrenergic receptors (147, 154), or vasoace intestinal peptide (VIP) (449). The involvement of e products of inositol phospholipid hydrolysis in the tentiation by H_1-receptor agonists of the cAMP reonse to adenosine was also studied using phorbol esrs and a calcium ionophore, but no clear conclusion uld be drawn (157, 322, 323).

Baclofen and other $GABA_B$-receptor agonists pontiate the cAMP response induced in rat cortical slices HA; however, the HA receptor subclass involved was t identified (380). Similar amplifications are found on imulation of α_1-adrenergic receptors, also linked with e phosphatidylinositol cycle (153, 449).

These amplification processes might be functionly important, inasmuch as they allow enlarged reonses to a single transmitter in increasing concentraons as well as mutual potentiations of the responses iggered by two neurotransmitters, e.g., HA and norepiphrine, reaching simultaneously the same target cell. ie potentiation between HA and adenosine might also functionally important in view of a possible corelease these substances from the same tuberomammillary lls.

Glycogenolysis

Although less abundant than in liver or muscle, ycogen represents in brain one of the major energy serves (at least 25% of the total) and may therefore be tal for meeting the high energy expenditure of neurol activity in the CNS. It is found not only in glial but so in neuronal cells (345). In brain the polysaccharide ores appear to be in a high dynamic state and to be ntrolled by several neurotransmitters (666). Cerebral ycogenolysis is studied in vivo in the neonatal chick, hich lacks a blood-brain barrier (203, 479), or, more nveniently, on brain slices in which levels of [^{3}H]glygen (synthesized from [^{3}H]glucose) are monitored usg a simple assay (590, 595). Glycogenolysis is triggered norepinephrine (203, 448, 480, 593, 595), histamine 0, 592, 595), adenosine (595), VIP (448), or 5-HT (594). ie effect of these various agents is due to phosphorylse activation in the slice preparation (790), which may cur either via cAMP accumulation, e.g., for responses ediated by β-adrenergic (593, 595) or VIP receptors 48), or via translocation of Ca^{2+}, which may activate osphorylase kinase by binding to its calmodulin subnit (135). The latter mechanism seems to apply to the lycogenolytic response to HA that requires Ca^{2+} in the ternal medium and is strictly mediated by H_1 receprs (590). An interesting feature of the HA response is iat it apparently occurs in a manner similar to that ediated by β-adrenergic receptors (593) with a large eceptor reserve (24, 590), which is consistent with the large signal amplification provided by the glycogenolytic cascade. In addition, rapid, selective, and rather large desensitization of the HA response, accompanied by limited changes in [^{3}H]mepyramine binding, can be observed in this system (592).

6. Guanosine 3',5'-cyclic monophosphate accumulation and other biochemical responses

Activation of guanylate cyclase and of inositol phospholipid hydrolysis appears to be a related cellular event (66, 318), which may explain why H_1 receptors mediate the HA-induced stimulation of guanosine 3',5'-cyclic monophosphate (cGMP) formation in mouse neuroblastoma cells (609, 611, 738). This response, similar to others mediated by H_1 receptors, requires an intact cell preparation and is Ca^{2+} dependent, suggesting that it is mediated by an increase in intracellular Ca^{2+}, but the latter could not be shown using the fluorescent probe aequorin (695a). In the same preparation, H_1-receptor stimulation triggers arachidonic acid release, possibly via activation of phospholipase A_2, which may indirectly be responsible for the cGMP response (695a). Stimulation of H_1 receptors in blocks of bovine sympathetic ganglia also increases cGMP accumulation (712). Finally, HA-induced cGMP responses were reported using brain slices (153, 425), but the responsible receptor subtype (presumably H_1) was not characterized. Histamine increases [^{3}H]norepinephrine release from brain slices, apparently via H_1-receptor stimulation (717). However, it increases endogenous norepinephrine release via a nonreceptor mechanism (836a).

7. Electrophysiological responses

Despite many studies of electrophysiological responses elicited by HA in CNS preparations (for reviews see Refs. 275, 276, 615), those mediated by H_1 receptors are generally not well defined, and their underlying membrane mechanism is poorly understood. One reason is that no highly selective H_1-receptor agonist is available and that many H_1-receptor antagonists have local anesthetic properties, which makes the interpretation of their effects often difficult. Nevertheless, it appears that iontophoretic application of HA to cerebral neurons often leads to excitatory responses when mediated by H_1 receptors. In vivo, osmosensitive neurons in cat supraoptic nucleus, also identified by antidromic invasion from the hypophyseal stalk, increase their firing rate when HA is applied, and this effect is blocked by mepyramine (281, 285). Whereas in cultured supraoptic nucleus neurons no effect of HA was observed (631), excitatory actions of HA, rather well characterized as being H_1 receptor mediated, were observed on acutely prepared explants of the same neurons (19). These excitations mainly occurred in cells either spontaneously active or in which burst activity had been induced antidromically, indicating that they were dependent on electrical activity expressed by the neurons. Although

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Review Article

Developments of histamine H_3-receptor antagonists

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CONTENTS

Introduction

The mediator and neurotransmitter histamine acts not only via the well-known H_1 and H_2 receptors (1) but also via a third type of receptor, termed H_3, which was described for the first time by Arrang, Garbarg and Schwartz in 1983 (2). This latter receptor differs from the H_1 and H_2 receptors in that, as a rule, it is not located on the effector cell (*e.g.*, on a smooth muscle cell) but "proximally" on neurones or paracrine cells. In detail, it may be located presynaptically on the axon terminals of histaminergic neurones (presynaptic autoreceptor; Figs. 1 and 2) or non-histaminergic neurones (presynaptic heteroreceptor; Fig. 2). Activation of the H_3 receptor causes inhibition of release of the respective neurotransmitter. The H_3 receptor may also be located on paracrine cells, *e.g.*, mast cells, enterochromaffin and enterochromaffin-like cells. Activation, again, causes inhibition of release of the respective mediators. The following is an overview of the occurrence of H_3-receptors on histaminergic and non-histaminergic neurones and on paracrine cells (3, 4).

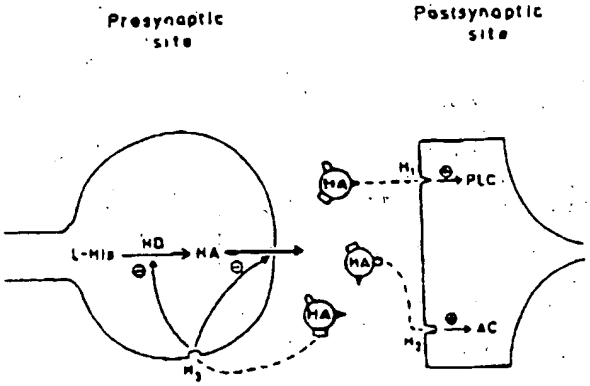

Fig. 1. Schematic drawing of the synapse between a histaminergic axon terminal and the subsynaptic membrane of an adjacent neurone. Note that histamine (HA) released exocytotically from the histaminergic axon terminal into the synaptic cleft activates H_1 and H_2 receptors, located postsynaptically, and the H_3 receptor, located presynaptically. H_1- and H_2 and most probably also H_3 receptors are coupled to G-proteins. The second messenger pathways indicated for the H_1 and H_2 receptors are not the only ones used by these two receptors. For the H_3 receptor, little information is so far available with respect to the second messenger (probably negative coupling to phospholipase C [PLC]). For further details regarding the molecular events following activation of the three histamine receptor subtypes see Schwartz *et al.* (3) or Leurs *et al.* (4). AC adenylyl cyclase; HD, histidine decarboxylase; L-His, L-histidine; +, stimulation; -, inhibition.

Unlike H_1- and H_2-receptor antagonists, which are widely used for the treatment of allergic diseases and gastroduodenal ulcers, respectively (1), H_3-receptor antagonists have not yet been marketed. The second part of this review gives an overview of recently synthesized H_3-receptor antagonists and their potential indications (5, 6).

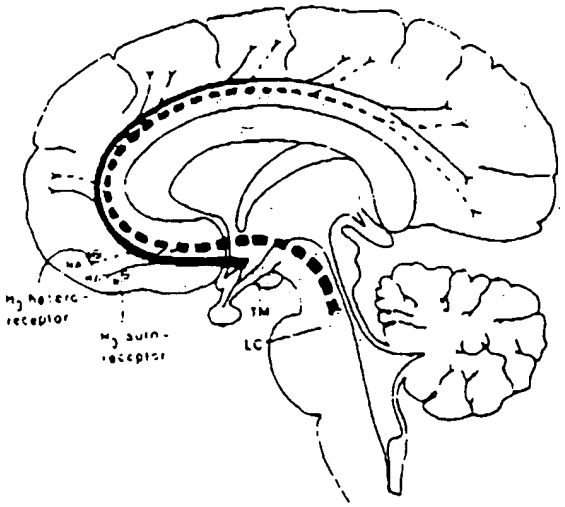

Fig. 2. Presynaptic inhibitory histamine H_3 auto- and heteroreceptors in the cerebral cortex of the human brain. The histaminergic neurones (solid line) originate in the tuberomammillary nucleus (TM) of the hypothalamus whereas the noradrenergic neurones (broken line) originate in the locus coeruleus (LC) of the pons. Both systems project to the cerebral cortex (note that this schematic drawing does not intend to give an exact representation of the respective tracts and projection areas) and to other parts of the CNS. The H_3 receptors involved in the inhibition of the release of histamine (HA) itself are referred to as "autoreceptors" whereas those involved in the inhibition of the release of another neurotransmitter (noradrenaline, NA) are termed "heteroreceptors". The occurrence of H_3 auto- and heteroreceptors in the human cerebral cortex has been shown by Arrang *et al.* (11) and Schlicker *et al.* (15 and unpublished data), respectively, although in both cases the presynaptic location was not proven.

Function of H_3 receptors

Presynaptic H_3 autoreceptors

The H_3 receptor described first (2) is an example of an H_3 autoreceptor. Arrang *et al.* (2) examined whether the histaminergic neurones, like the noradrenergic, serotonergic or cholinergic neurones (7), are endowed with an autoreceptor via which the respective transmitter modulates its own release. They studied the potassium- or veratridine-induced release of [^{3}H]-histamine (formed [and subsequently separated] from [^{3}H]-histidine) in rat brain cortex slices. Histamine inhibited the evoked [^{3}H]-histamine release; however, the pharmacological properties of this effect did not conform to H_1- or H_2-receptors. Thus, the effect of histamine occurred in a very low concentration range, was not mimicked by the H_1-receptor agonist 2-(2-thiazolyl)ethanamine or the H_2-receptor agonist dimaprit, and was not antagonized by low concentrations of the H_1-receptor antagonists mepyramine and (+)-chlorpheniamine or the H_2-receptor antagonists cimetidine and ranitidine. On the other hand, the H_2-receptor antagonist burimamide and the partial H_2-receptor agonist impromidine proved to be competitive antagonists at this new histamine receptor at potencies even higher than those at H_2 receptors (2). The latter drugs, by themselves, increased the evoked [^{3}H]-histamine release by interrupting the local feedback built up by endogenous histamine in the slices.

In the subsequent years, the H_3 autoreceptor was also identified by other investigators (8, 9). Moreover, its presynaptic location was proven (10), and its occurrence in other brain regions of the rat (hippocampus and striatum; 10) as well as in the cerebral cortex of humans (11) was shown. The H_3 autoreceptor also operates *in vivo* (12, 13); furthermore, activation of the histamine H_3 autoreceptor not only inhibits the release of histamine but also its synthesis from L-histidine (14) (Fig. 1).

Presynaptic H_3 heteroreceptors

Since 1987, histamine H_3 heteroreceptors have been identified on non-histaminergic neurones in the central nervous system (CNS) and on sympathetic, parasympathetic and nonadrenergic noncholinergic nerve fibers supplying the gastrointestinal, bronchial and/or cardiovascular system (Table I). H_3 heteroreceptors also operate *in situ* or *in vivo* (15-17, 26, 27, 31-33) and also have been identified (under *in vitro* conditions) in the human CNS, saphenous vein (15), heart (29) and segmental bronchi (25).

However, presynaptic H_3 heteroreceptors do not generally occur on neurones; there are, *e.g.*, regional and species differences. Thus, the sympathetic nerve fibers innervating the resistance vessels of the rat are endowed with H_3 receptors, whereas the sympathetic neurones supplying the vena cava of this species are not (15). Moreover, the cholinergic neurones innervating the cat or dog stomach (16, 17), unlike those innervating the rat stomach (34), appear to be endowed with H_3 receptors. Some of the H_3 heteroreceptors, *e.g.*, that in the cat (16), are activated by endogenous histamine as well since H_3-receptor antagonists facilitate this effect. For others, however, a physiological role is less likely since histamine H_3-receptor antagonists did not facilitate the release of the respective transmitter and/or the extent of the inhibitory effect of H_3-receptor agonists was small. Some H_3 heteroreceptors may be activated under pathophysiological conditions. Thus, an H_3-receptor antagonist markedly increases noradrenaline release in guinea pig hearts which have undergone ischemia but fails to do so in control hearts (28).

H_3 Receptor on paracrine cells

Since the histaminergic neurones in the CNS are endowed with H_3 autoreceptors, it was tempting to examine whether such receptors also occur on mast cells which, outside the CNS, represent the major source of histamine. In rat peritoneal mast cells, the anaphylactic histamine release was inhibited by histamine and H_3-receptor agonists (35, 36). On the other hand, such an effect was not obtained for the concanavalin A-induced histamine release from human adenoidal mast cells (37) and for the formyl-methionyl-leucyl-phenylalanine- or anti-IgE-induced histamine release from human basophils (38). In the study of Dimitriadou *et al.* (39), evidence for a feedback loop involving mast cells plus C fibers was presented for the rat lung and spleen. Mast cells and C fibers are in close apposition. The C fibers, which are nonadrenergic, noncholinergic neurones (Table I), are endowed with H_3 receptors; activation

Table I: Occurrence of histamine H_3 heteroreceptors in the CNS and autonomic nervous system.

	Type of neurone	Species	Region (of CNS), organ or tissue	Ref.
CNS	Noradrenergic	Rat	Cortex, hypothalamus	15
		Mouse, guinea pig, rabbit, human	Cortex	15
	Cholinergic	Rat	Entorhinal cortex, ventral striatum	15
	Dopaminergic	Mouse	Striatum	15
	Serotonergic	Rat	Cortex, striatum, hypothalamus	15, 48
Gastrointestinal tract	Cholinergic	Cat, dog	Stomach	16, 17
		Guinea pig	Jejunum, ileum, enteric ganglia, longitudinal muscle myenteric plexus	18-21
	NANC	Guinea pig	Duodenum, jejunum, ileum, colon	22, 23
Bronchial tract	Cholinergic	Guinea pig	Trachea	24
		Human	Segmental bronchi	25
	NANC	Guinea pig	Trachea, bronchi	26
Cardiovascular system	Noradrenergic	Rat, guinea pig, human	Heart	15, 27-29
		Rat	Resistance vessels	15
		Guinea pig	Mesenteric artery, submucosal arterioles of ileum, tracheobronchial postcapillary venules, resistance vessels	15, 27, 30, 31
		Pig	Retinal vasculature	15
		Human	Saphenous vein	15
	NANC	Rat	Dura mater, postcapillary venules	32
		Guinea pig	Tracheobronchial postcapillary venules	33

Note that the presynaptic location has not been proven for each H_3 receptor in the table. Moreover, some H_3 receptors in the autonomic nervous system were identified using an indirect approach, thus, the influence of H_3-receptor ligands on the end-organ response elicited by an endogenously released neurotransmitter, rather than on the release of this transmitter itself, was determined. Refs. 48 and 16-33 refer to original papers not yet considered in the review by Schlicker *et al.* (15). NANC, nonadrenergic, noncholinergic, neurotransmitter in this type of neurone: substance P

of which inhibits the release of substance P and calcitonin-gene-related peptide. The two peptides in turn increase histamine release from mast cells. Dimitriadou *et al.* found that H_3-receptor agonists and antagonists decreased and increased the synthesis of histamine in the two organs, respectively. These effects did not occur if the rats (at the age of 2 days) were treated with capsaicin which is known to cause degeneration of C fibers.

Two types of paracrine cells in the gastrointestinal tract are endowed with H_3 receptors. Thus, serotonin release from the enterochromaffin cells of the porcine intestine is inhibited via H_3 receptors (40). Furthermore, an H_3-receptor-mediated inhibition of histamine release in the stomach of the rabbit, most probably from the enterochromaffin-like (ECL) cells, was shown (41). It is an attractive hypothesis that H_3 receptors, both on cholinergic neurones (Table I) and on ECL cells, may represent a "brake" which counteracts an excessive increase in gastric acid output. It is conceivable that H_3 receptors also subserve this function in humans since H_3 receptors were found in a human gastric cell line (42).

History of H_3-receptor antagonists

The history of histamine H_3-receptor antagonists dates back to the year 1973 when Ambache *et al.* (43) found that the H_2-receptor antagonist burimamide (Fig. 3) counteracted the inhibitory effect of histamine (studied in the presence of the H_1-receptor antagonist mepyramine) on the neurogenic contraction of the guinea pig ileum. This effect was later shown to involve H_3 receptors (22, 23). Burimamide and the H_2-receptor agonist impromidine (Fig. 3) were used in the study of Arrang *et al.* (2), in which the H_3 autoreceptor was described for the first time (see above). Mifentidine, another H_2 receptor ligand bearing an unmethylated imidazole moiety (like burimamide and impromidine), also proved to be a potent histamine H_3-receptor antagonist, whereas H_2-receptor antagonists with a methylated imidazole ring (*e.g.*, cimetidine or metiamide) or without this heterocycle (*e.g.*, ranitidine or zolantidine) had low or negligible potency at H_3 receptors (2, 44). A low, or at best moderate, antagonist potency at H_3 receptors is also displayed by betahistine (45), an H_1-receptor agonist marketed for the treatment of vestibular disturbances such as Ménière's disease, as well as the psychotomimetic drug phencyclidine (46) and the atypical neuroleptic clozapine (47, 48).

Potent and selective histamine H_3-receptor antagonists were synthesized from 1986 on (49); thioperamide (Fig. 3), described by Arrang *et al.* (12) and widely used in experimental studies, is still the reference compound. Although thioperamide and the isothiourea derivative clobenpropit are highly potent compounds, they have never been reported to be used in clinical trials. Most probably pharmacokinetic problems, side effects and/or high affinity to other G-protein-coupled receptors (50) are the reasons for the lack of development. Therefore, novel compounds displaying high activity *in vitro* and *in vivo* as well as high selectivity are highly recommended.

Chemistry

The most crucial point in the design of novel H_3-receptor antagonists is their imidazole moiety. It is carried out with much effort over ring closure reactions, and most of the time

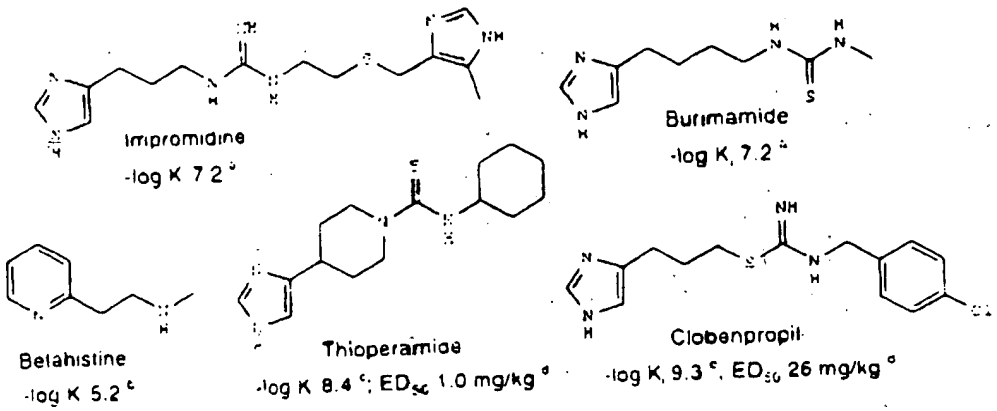

Fig. 3. Histamine H_3-receptor antagonists. Ref.: a (2), b (45), c (12), d (67), e (102).

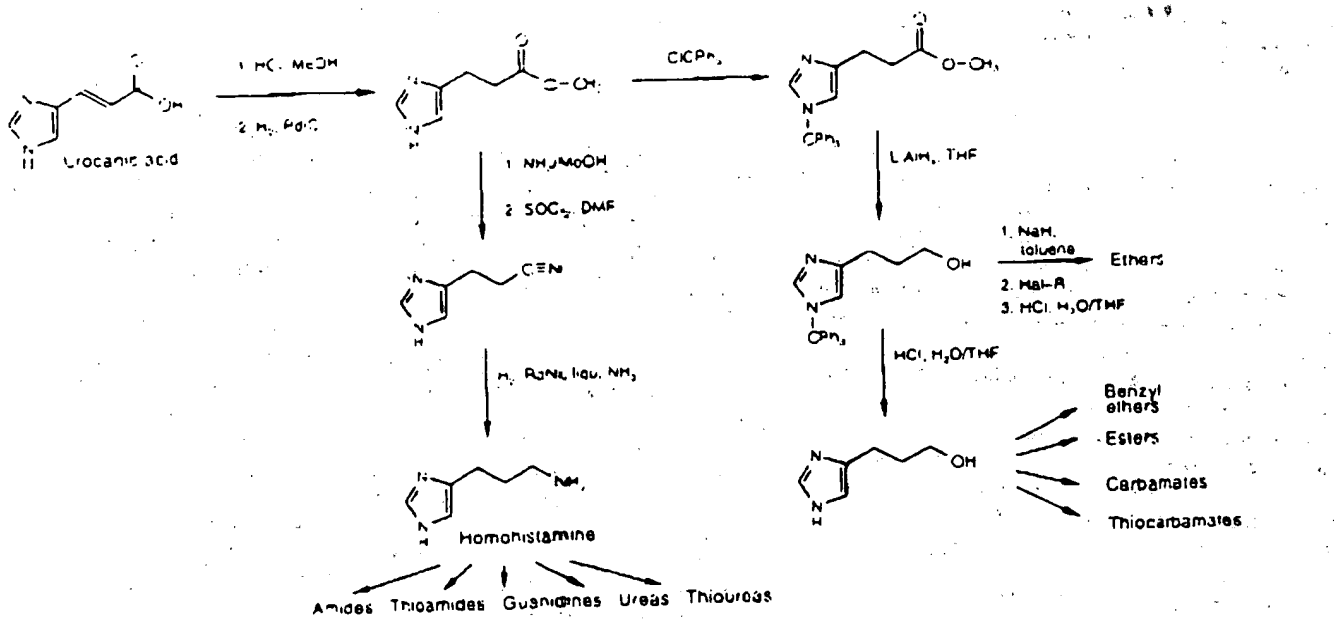

Fig. 4. Synthesis of versatile synthons for H_3-receptor antagonists.

imidazoles are obtained with low yields and many side products (51-54). Industrial synthesis used to prepare large amounts of a single synthon does not influence drug development in the first stage (55). Therefore, different syntheses have been developed to take protected imidazole or substituted derivatives as educt in efficient drug design, e.g., 2-(*tert*-butyldimethylsilyl)-1-(*N,N*-dimethylsulfamoyl)-imidazole for alkylation in 4-position (56).

For the preparation of 4-propyl substituted imidazole derivatives *trans*-urocanic acid (3-(4-(1*H*)-imidazole)propenoic acid) is a convenient synthetic tool (Fig. 4). Urocanic acid itself was produced from histidine in a microbiological synthesis (57). It is converted into methyl ester and hydrogenated on Pd/C to methyl 3-(4-(1*H*)-imidazole)propionate. In liquid ammonia/methanol this intermediate can be converted into the corresponding amide. Dehydratization with thionyl chloride and subsequent catalytic hydrogenation of the nitrile with Raney nickel in ammonia under high pressure led to homohistamine, a homologous compound of the biogenic amine histamine. All reaction steps were performed on a large scale with high yields (75-95%) and low amounts of side products (58). This amine was used as a versatile synthon for further derivatization (59). Amides were prepared by this synthon with acyl halides under Schotten-Baumann procedure (60, 61). Reduction of these amides with $NaBH_4$ after activation with $POCl_3$ led to secondary amines (60).

For the synthesis of impromidine an isothiourea precursor was reacted with homohistamine leading to the guanidino compound (62). Unsymmetrically di- and trisubstituted guanidines were comfortably prepared by the reaction of primary amines with diphenylimidocarbonate derivatives. A

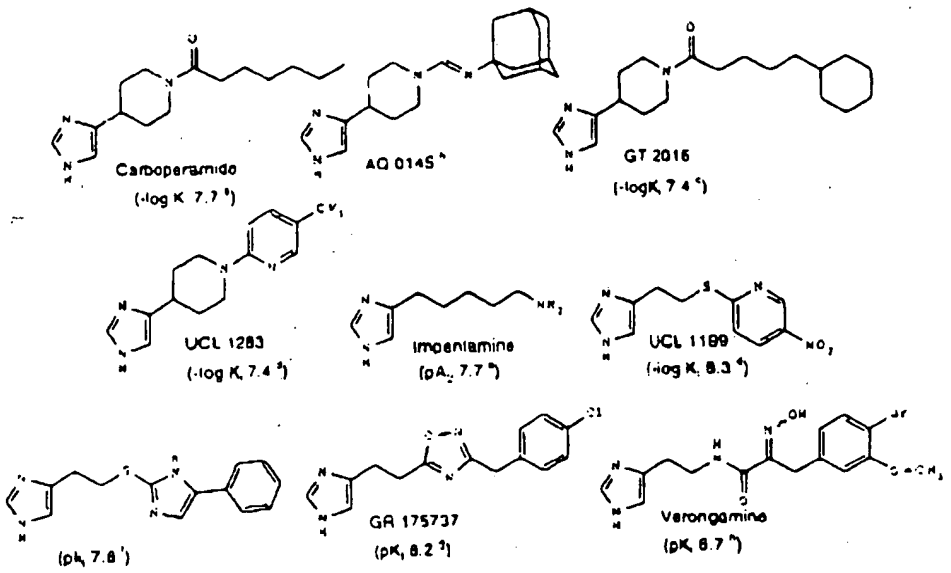

Fig. 5. Miscellaneous developments of H_3-receptor antagonists. Ref.: a (102), b (77), c (76), d (64), e (79), f (78), g (82), h (83).

sequential displacement of both phenoxy groups led to electron-withdrawing trisubstituted guanidines which could be easily purified and hydrolyzed to unsymmetrically disubstituted guanidines (63). Reaction with isocyanates and isothiocyanates resulted in urea and thiourea derivatives, respectively, structurally related to burimamide (64, 65). All these amine derivatives have proved to be potent H_3-receptor antagonists.

Methyl 3-(4-(1*H*)-imidazole)propionate could not exclusively be converted into homohistamine and related derivatives. Only a short overview of derivatization possibilities can be given in this report. After imidazole protection by tritylation the ester was converted into the corresponding alcohol by complex hydrides like $LiAlH_4$ in THF (66) (Fig. 4). Tritylation increases the yield of this reduction and facilitates purification. By this procedure the alcohol synthon was obtained in imidazole protected and unprotected form in high purity. The bivalent functionality of imidazolylalkyl alcohol is often a problem with further derivatization.

The hydrochloride salt of the untritylated alcohol is reacted with isocyanates to result in the carbamate derivatives (67). Due to the salt form the nucleophilicity of the imidazole ring is reduced, and the hydroxy functionality is the only point of attack. The protonation of the imidazole nucleus is preferred to a possible protection by tritylation or comparable protective groups because most cleavage conditions of protecting groups would also result in a hydrolysis of the carbamate moiety. Benzyl chlorides or related structures are reactive electrophilic agents. The salt of the imidazole alcohol could directly be reacted with these halides in order to result in high yields of ethers (68). Acetonitrile is the best solvent for this reaction because when warmed it is able to dissolve the hydrophilic salt as well as the lipophilic halides.

Tritylated alcohol is a convenient synthon for Williamson ether synthesis to react in the form of alcoholate with different alkyl halides (69). The addition of crown ether increases yields. The inverse synthesis is also possible with the conversion of the tritylated alcohol by thionyl chloride into propyl chloride derivatives and subsequent reaction with alcoholate ions which may be – in the form of their halides – sensitive in alkaline solution to elimination and following side reactions (70).

Structure-activity relationships and general pattern

The activity values given in this text are based on K^+-evoked depolarization-induced release of [^{3}H]-histamine from rat synaptosomes for *in vitro* tests (71) and on an increase in the level of the histamine metabolite N^{π}-methylhistamine in brain after *per os* application to Swiss mice for *in vivo* activity (71). All data based on other models are given in parentheses.

The first step for the design of highly potent and selective H_3-receptor antagonists was done by the development of thioperamide (49) (Fig. 3). The imidazolylpiperidine moiety (72) was the starting point for many research groups for further development.

Miscellaneous developments

Therefore, it is not astonishing that a lot of new derivatives like ureas, amides or amidines are based on that structural element (Fig. 5). The Robba and Schwartz groups who had developed thioperamide designed a related heptanamide derivative, carboperamide (73) (Fig. 5). A comparable cyclohexane-5-pentanamide (GT 2016) was later prepared by Durant and co-workers (74, 75) and developed in the USA by Gliatech (76). The Japanese Green Cross Corporation (77) investigated an adamantane-containing amidine derivative (AQ 0145) (Fig. 5) which seems to be structurally related to mifentidine, an H_2/H_3-receptor antagonist (44). Ganellin and co-workers (64) introduced a heterocyclic moiety as substituent of the partial thioperamide structure.

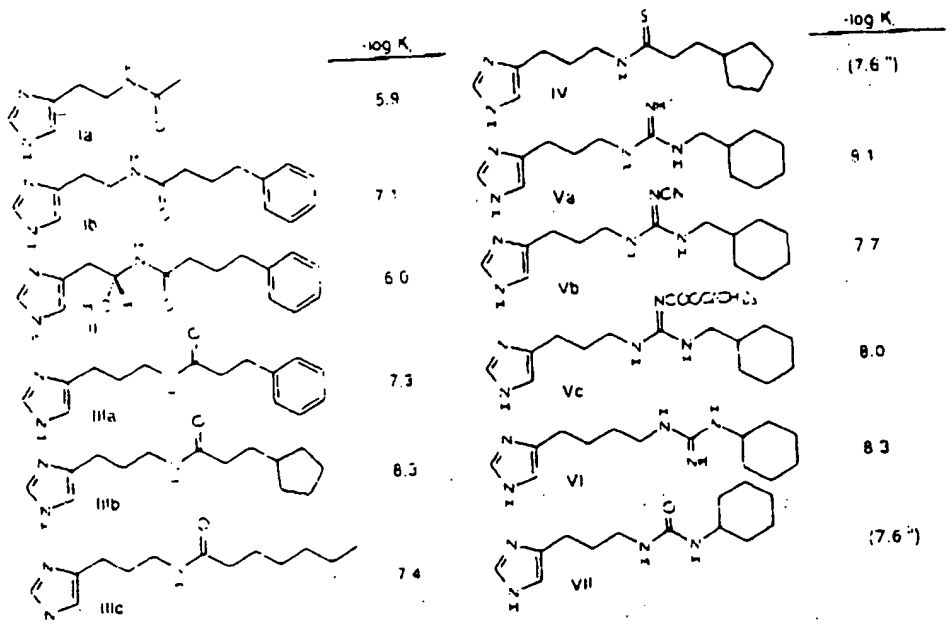

Fig 6. N^{ω}-Functionalized ω-(1*H*-imidazol-4-yl)alkylamine derivatives. Ref.: a (81).

the rigid piperidine moiety (UCL 1283) (Fig. 5). This approach was also successful with structurally less restricted alkyl chains, *e.g.*, 2-(1*H*-imidazol-4-yl)ethyl thioether derivatives and was used by different groups (64, 78) (Fig. 5).

Different heterocyclic moieties could be introduced while maintaining H_3-receptor antagonist activity. The independence of a large number of different substituents and their positions on the heterocycles indicate that additional affinity is contributed through hydrophobic interaction more than by hydrogen bonding. In investigations on the alkyl chain it was found that side chain elongation of histamine changes agonist to mostly antagonist behavior and resulted in impentamine (79) (Fig. 5). The 4-monosubstituted imidazole ring connected to an alkyl chain seems to be responsible for H_3 receptor binding where in most classes a three methylene residue was found as an optimum. Although some nonimidazoles such as betanistine (45) (Fig. 3), phencyclidine (46), dimaprit (44, 80) and clozapine (47, 48) have some antagonist activity, they are much less potent than imidazole derivatives. On the other hand, a large variety of structures are accepted by maintaining H_3 receptor activity at the connecting polar group. The diversity of heterocyclic or lipophilic moieties does not point to specific receptor binding. A heterocycle could also be introduced directly into the imidazolylalkyl chain by maintaining the activity as shown with oxadiazole derivatives such as FUB 220 (96) and GR 175737 (82) (Fig. 5).

Only very little *in vivo* data of all compounds is available, and in most cases could not be compared to each other. A direct comparison of thioperamide, clobenpropit and GR 175737 was made concerning their *in vivo* activity after s.c. application to rats for agonist-induced dipsogenicity showing respective ED_{50} values of 0.8 mg/kg, 1.3 mg/kg and 1.0 mg/kg, and for *ex vivo* binding of [^{3}H]-N^{ω}-methylhistamine mg/kg, and 1.2 mg/kg, respectively (82). A compound of special interest is verongamine (83, 84) (Fig. 5). This moderately active H_3-receptor antagonist representing an amide derivative of histamine was extracted from the marine sponge *Verongula gigantea*. Very recently a series of substituted 4-(phenylmethyl)-1*H*-imidazoles have been described as potent H_3-receptor antagonists (85).

Amine derivatives

The activity of verongamine (Fig. 5) is in accordance with the observation that N^{ω}-acetylhistamine (Ia, Fig. 6) was also found to be a moderate antagonist (60). Optimization of this starting point led to a series of ω-(1*H*-imidazol-4-yl)alkyl amine derivatives (Fig. 6). In a series of amide derivatives the different alkyl spacers (chain A, chain B; see Fig. 7) were varied. In the series of histamine amides a 4-phenylbutanoic acid derivative (Ib) was found to be one of the most active histamine derivatives (60). Interestingly, the analogous amide (II) of the reference agonist (*R*)-α-methylhistamine is clearly less effective than the related amide of histamine. This finding indicates an antagonist receptor binding distinctly different from that of agonists. Taking into account the development of impentamine

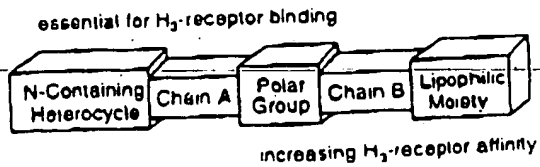

Fig. 7. General construction pattern for histamine H_3-receptor

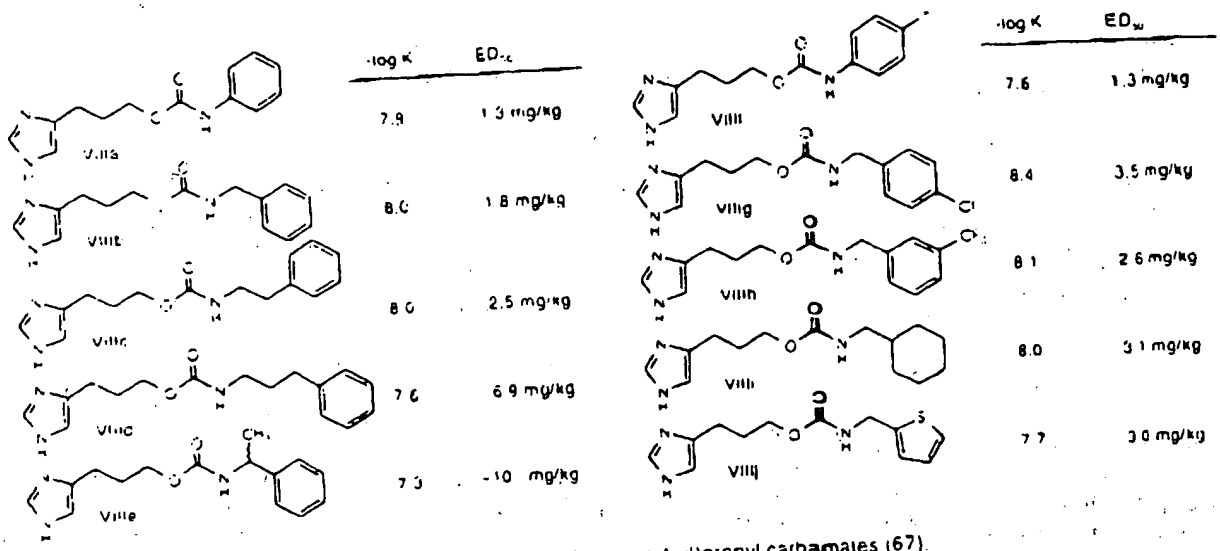

Fig. 8 *N*-Substituted 3-(1*H*-imidazol-4-yl)propyl carbamates (67).

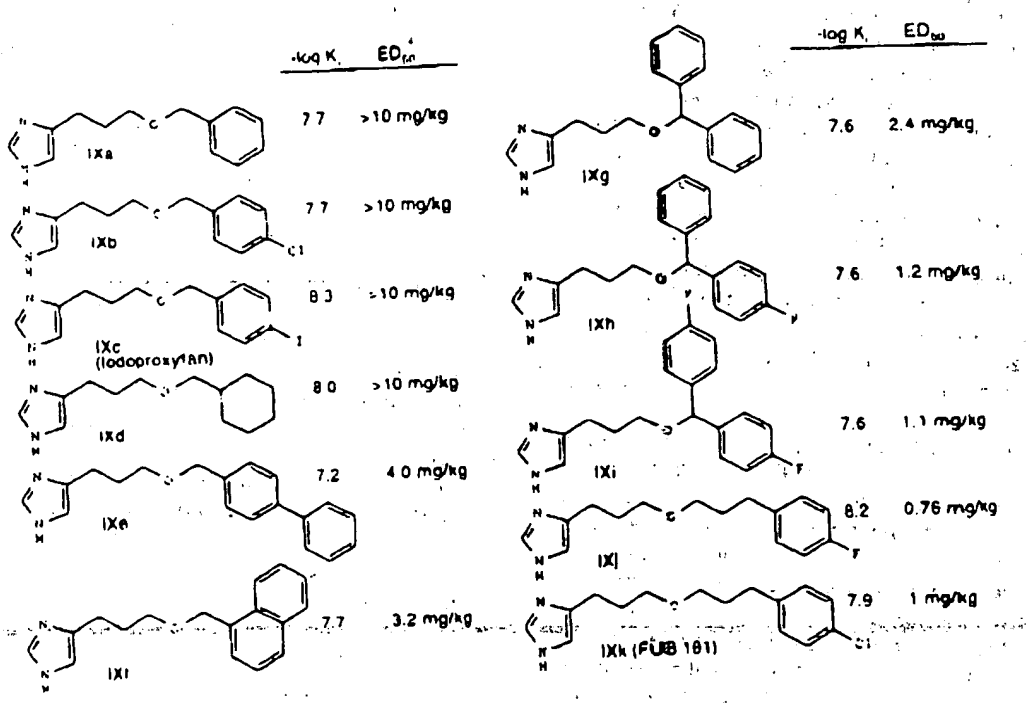

Fig. 9 4-(3-(ω-(Aryl)alkyloxy)propyl)-1*H*-imidazoles.

pharmacokinetic need. Having a clear therapeutic indication for H_3-receptor antagonists absorption, half-life, metabolism and other factors can be influenced by different substitution patterns while maintaining the *in vivo* activity. This is the main advantage of this potential class of drugs.

Ethers

Further exchange of heteroatoms in the polar group led to esters and ethers. Although esters showed high potency *in vitro* (81, 96), their missing *in vivo* activity was the reason for not pursuing this class of compounds in more detail (66). Most probably the instability of these compounds against the attack of unspecific esterases in blood may be responsible for this inactivity. Although it might be possible to increase metabolic resistance it was found more promising to investigate the ether class. Metabolic stability was expected to be much higher compared to that of esters. Therefore, we were astonished that most benzyl ethers or related derivatives proved to be highly active *in vitro*, but almost inactive *in vivo* (IXa-d, Fig. 9). Only some compounds with voluminous aryl substituents (IXe, f) showed *in vivo* activities below 10 mg/kg after peroral application to mice. It might be speculated that the attack of hydrolyzing enzymes is hindered by these bulky substituents, but other

this finding is not obvious. Variation of the histamine moiety led to a series of homohistamine amides in which activity could be plainly increased (IIIa-c Fig. 6) (61). Compared to the reference antagonist thioperamide, the cyclopentane-3-propanamide IIIb is active in the same concentration range. Comparable lipophilic residues (*e.g.*, IIIc) as in thioperamide (Fig. 3) or carboperamide (Fig. 5) led to lower activity.

Based on these findings and on the knowledge of other classes of histamine H_3-receptor antagonists, we developed a general construction pattern shown in Figure 7 which fits to almost all potent H_3-receptor antagonists (5, 59). In most cases, the nitrogen-containing heterocycle is an imidazole ring monosubstituted in 4-position. It seems that other moieties could imitate the imidazole, but up to now this change has been linked to a loss in activity of at least two orders of magnitude. Intense investigations on the replacement of or changes on 4-imidazolyl moiety drastically showed the importance of this heterocycle (60, 61, 64, 86-88). Almost any substitution or change to other 5- or 6-membered heterocycles led to a loss of or an immense reduction in affinity. In most classes of histamine H_3-receptor antagonists, three methylene groups were found to be the optimum for chain A, but this is not the case for all classes. The diversity of the polar group is the most notable element in this common structure. Basicity is of minor importance because amines and guanidines are active in the same range as amides, thioamides and other non-basic classes. Especially trisubstituted guanidines (Vb, c, Fig. 6) containing electron-withdrawing groups are no longer basic and possess different electronic and steric properties depending on substitution. Although they are less active than the corresponding disubstituted guanidines (Va, VI, Fig. 6) they are still potent antagonists (63). Their main advantage is that they might be potential prodrugs for the disubstituted guanidines because basic guanidine derivatives like impromidine are ineffective with this way of application (89).

The hydrolysable group of the guanidino functionality may lead to an increased bioavailability of these compounds, *i.e.*, these trisubstituted guanidino derivatives may be well absorbed from the gastrointestinal tract. The disubstituted guanidines formed in the body from the trisubstituted ones may be distributed well into peripheral tissues. As highly hydrophilic compounds, they are not expected to cross the blood-brain barrier. Thus, trisubstituted guanidines of that kind might represent prototypes of H_3-receptor antagonists with an effect restricted to the periphery. Since the development of a reliable *in vivo* test system for peripheral H_3-receptor antagonists is still in progress (90), this hypothesis could not be proved up to now. With respect to the reference H_3-receptor antagonist thioperamide, penetration through the blood-brain barrier is discussed controversially (91, 92).

Variation on the polar group of homohistamine derivatives could also result in N-cyclohexyl thiourea (64, 65) or *N*-cyclohexyl urea derivatives (VII, Fig. 6) (81) strongly related to thioperamide. These compounds are only slightly less potent than thioperamide demonstrating that the three-methylene chain could easily imitate the alkyl chain of the piperidine moiety. Only in the case of the *trans*-configured isomer introduction of a double bond in chain A led to a compound active in a concentration range comparable to the parent compound (61), giving a hint for the steric conformation of the antagonist when interacting with the receptor.

The construction pattern is a generalized structure which shows many variables of histamine H_3-receptor antagonists. Modern computational methods have just begun to do this in a more rationalized way (93-95). Although these studies brought useful aspects in the discussion on structural conformations it was not possible to build a conformer or to predict any binding amino acid sequence for histamine H_3-receptor antagonists. It is expected that in the near future further molecular modeling studies combined with the results of receptor cloning will bring more knowledge to ligand-receptor molecular interaction.

All the compounds presenting high potencies have in common that their polar group is sulfur-containing or is possibly able to build a large number of hydrogen bonds. On the one hand, sulfur moieties often seem to be associated with side effects *in vivo* (hepatotoxicity, changes in blood picture, influence on thyroid hormones, *etc.*). On the other hand, the high number of hydrogen bonds decreases the penetration through the blood-brain barrier, and therefore the target area with the highest density of H_3 receptors is reached to a much smaller extent.

The aim of further investigations was focused on improved central *in vivo* activity without any predictable toxicity of the compounds. Pharmacokinetic behavior should also be taken into account.

Carbamates

For an enlargement of side chain functionalized imidazole alkyl derivatives oxy analogous compounds were prepared as new leads (59). Compared to ureas, thioureas and guanidines, heteroatoms were replaced by oxygen in the new carbamate class (Fig. 8). Carbamate derivatives are a class of histamine H_3-receptor antagonists with strikingly homogeneous *in vitro* activities differing less than one order of magnitude (66, 67, 96). Only a limited number of steric or hydrophilic factors must be taken into account concerning the development of this class. This lack of discrimination of chirality (VIIIe, Fig. 8) (67), of different substituents and of different positions (VIIIf-h, Fig. 8) shows that these structural elements are not involved in a close ligand-receptor molecular interaction. In the class of isothioureas like clobenpropit (Fig. 3) a *p*-chloro substituent at the benzyl group representing the lipophilic moiety of the general construction pattern (Fig. 7) increases the activity more than one log-unit (97). In the related carbamate class similar structure-activity relationships could not be confirmed giving strong evidence for a different receptor binding of both chemical classes of H_3-receptor antagonists.

Although the carbamates do not reach the activity level *in vitro* of clobenpropit, a large number of carbamate derivatives are much more effective *in vivo*. Especially the high number of chemical variations (Fig. 8) concerning the lipophilic moiety (aryl, heteroaryl [VIIIj], cycloalkyl [VIIIi] residues), including the spacer (chain B) (VIIIa-e), open chemical possibilities of drug targeting corresponding to the

Table III. Affinity and antagonistic potency of clozapine and its two major metabolites at H_3 receptors and concentrations of clozapine obtained in humans during treatment

		Clozapine	*N*-Desmethyl-clozapine	Clozapine-*N*-oxide
Affinity	pK_i	6.2[a]; 6.6[b]	5.3[c]	4.2[b]
Potency	pA_2	6.3[c]; 7.1[d]	5.8[c]	4.2[c]
Free plasma concentration	-log mol/l	7.9[e]		
Calculated concentration in the brain	-log mol/kg	(6.5[f])		

[a]Determined on rat brain cortex membranes, ligand [^{3}H]-*N*$^{\alpha}$-methylhistamine (47,109). [b]Determined on rat brain cortex membranes, ligand [^{125}I]-iodoproxyfan (48). [c]Determined in a functional H_3 receptor model on mouse brain cortex slices (47,109). [d]Determined in a functional H_3 receptor model on rat brain cortex slices (48). [e]The weighted mean of several studies corrected for the plasma protein binding (95%) was used (from 119). [f]This value was calculated assuming that the level of clozapine in the human brain is 24-fold higher than in the serum (like in the rat; 110).

H_3-Receptor antagonists as drugs against schizophrenia?

Several lines of evidence suggest that histamine may play a role in the pathogenesis of schizophrenic diseases. Thus, the level of *N*$^{\alpha}$-methylhistamine, a metabolite of histamine was found increased in the cerebrospinal fluid of schizophrenic patients, whereas the concentration of metabolites of other neurotransmitters was not changed (107). Furthermore, the density of H_1 receptors in the postmortem frontal cortex of schizophrenic patients was reduced (108). Therefore, it was of interest to consider whether blockade of H_3 receptors is a thus far unknown property of typical and/or atypical neuroleptics and might be involved in their therapeutic effect.

In a series of 11 neuroleptics (including phenothiazines, butyrophenones and benzamides), only clozapine had a moderate binding affinity for H_3 receptors; subsequent functional experiments revealed that the compound behaves as a competitive antagonist at this receptor (47, 48, 109) (Table III). Clozapine, admittedly, possesses a high affinity for dopamine D_4, serotonin 5-HT_{2A} and muscarinic acetylcholine receptors (pK_i of about 8; see ref. 47), which may explain its atypical neuroleptic profile. Nonetheless, its antagonistic effect at H_3 receptors should also be considered in this context: If one assumes that (i) the affinity of clozapine for H_3 receptors is similar in the rat and human brain and (ii) the clozapine level in humans (like in the rat; 110) is 24-fold higher in the brain than in the serum, a concentration of clozapine similar to its affinity constant can be reached in the human brain (Table III). The possibility that one of the two major metabolites of clozapine has an even higher affinity/potency at H_3 receptors than clozapine itself was excluded (109) (Table III).

H_3-Receptor antagonists as antiepileptic drugs?

With respect to this potential indication, a few studies on animals are already available. Yokoyama *et al.* showed that the H_3-receptor antagonists thioperamide (111) and clobenpropit (112) inhibited electrically induced convulsions in mice. The effect of either antagonist was counteracted by the H_3-receptor agonist (*R*)-α-methylhistamine, demonstrating that H_3 receptors are involved. In addition, the effect of thioperamide or clobenpropit was counteracted by the H_1-receptor antagonist mepyramine, but not by the H_2-receptor antagonist zolantidine. The latter findings can be explained by the fact that due to the interruption of the tonical inhibitory effect exerted by endogenous histamine on the H_3 receptors (2, 11), an increase in histamine release is obtained.

Activation of H_1 receptors is known to increase the threshold for seizures (111-113) as evidenced by the anticonvulsant effect of L-histidine (the precursor of histamine) or metoprine (an inhibitor of the degradation of histamine). On the other hand, (*S*)-α-fluoromethylhistidine (an inhibitor of the formation of histamine) and H_1-receptor antagonists possess a proconvulsant effect. The latter also holds true for humans since H_1-receptor antagonists (antihistaminics) increase epileptic discharges and occasionally induce seizures in epileptic patients (111-113).

H_3-Receptor antagonists as cognition-enhancing drugs?

For this potential indication, again studies in animals are available. Favorable effects of the H_3-receptor antagonists thioperamide and GT 2016 on learning and memory have been obtained in behavioral studies in rodents (114-116, 120) (Table IV). This also holds true for rodent models in which learning and memory are not yet fully developed (juvenile rat pup; 114), or are impaired chemically (by diazepam and scopolamine; 117, 120), or on a genetic basis (senescence-accelerated mouse; 116) (Table IV). A role for histamine in learning processes has been shown in animals (113). Moreover, the level of histamine in the cerebrospinal fluid was found to be altered in humans suffering from Alzheimer's disease as compared to healthy controls (113); in the postmortem brain from patients with Alzheimer's disease the histaminergic neurones showed significant alterations (118).

Whether H_3-receptor antagonists are useful for the latter and for other indications has to be examined in future studies.

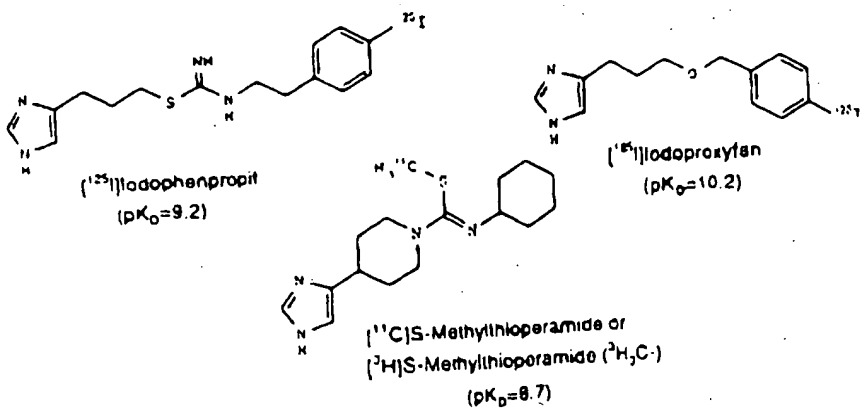

Fig 10 Radiolabelled H_3-receptor antagonists.

reasons like higher lipophilicity have also been taken into account. The diphenylmethyl ether derivatives IXg-i showed modest homogeneous activity *in vitro* but high activity *in vivo*. When these compounds were tested for their selectivity for H_3 receptors compared to other histamine receptor subtypes a clear H_1-receptor antagonist activity was also found. The structural accordance with classical H_1-receptor antagonists, *e.g.*, diphenhydramine, containing a lipophilic diaryl structure and a basic moiety in a distinct distance, could be the explanation for this unique pharmacological profile.

Variations of chain B brought additional progress in this lead structure. The halogen substituted 3-phenylpropyl ether derivatives IXj and IXk (FUB 181) belong *in vivo* to the most potent H_3-receptor antagonists reported so far. Once more a halogen substituent in *para*-position of the aryl moiety showed an activity increasing effect. Furthermore, the selectivity of these compounds compared to the diphenylmethyl derivatives has largely increased by these structural variations, *e.g.*, FUB 181 (IXk; at least 100:1 for H_3:H_1, H_2, α_1, β_1, 5-HT_{2A}, 5-HT_3, and M_3) (70).

Recently Ganellin and co-workers reported on a promising 4-(ω-(phenoxy)alkyl)imidazole class presenting high *in vitro* activity (98). Chain B was deleted without loss of *in vitro* activity, whereas a two- or three-methylene spacer for chain A was active in the same concentration range depending on the substitution pattern of phenyl moiety. To date, there is no report on *in vivo* activity of these compounds.

Radioligands

Tritium-labelled derivatives of *N*$^{\alpha}$-methylhistamine or the more selective ligand (*R*)-α-methylhistamine are state-of-the-art agonists for binding investigations. The problems of agonist receptor binding are not discussed in this article (see ref. 99), but radiolabelled antagonists are much more useful for most investigations concerning receptor binding. The first radiolabelled antagonist was the isothiourea derivative [^{125}I]-iodophenpropit (100, 101) (Fig. 10). Although it showed satisfying pharmacological properties, this radioligand displays some limitations (102), especially high affinity for 5-HT_3 receptors (50) and its unspecific binding properties (101). This led to an intense investigation for new iodinated H_3 receptor ligands which could be used in radiolabelled form for further pharmacological and molecular biological studies (68). The newly developed [^{125}I]-iodoproxyfan (Fig. 10) shows high activity, high affinity, high specificity, and saturable and reversible binding (102). When tested for activity at other related receptors like H_1, H_2, α_1, α_2, β_1, 5-HT_{2A}, 5-HT_3, and M_3 receptors, its high H_3 receptor selectivity was demonstrated (102, 103). The radiolabelling could be performed by nucleophilic copper(I) catalyzed exchange reaction using the corresponding bromo derivative as a precursor (66). The autoradiographic picture obtained in a short time showed distinguishable laminated patterns of labelling in the cerebral cortex demonstrating the sensitivity and the utilization of this commercially available radioactive probe (102).

Recently, the binding characteristics of [^{3}H]-labelled *S*-methylthioperamide (Fig. 10) were reported (104). This structure might also be used in [^{11}C]-labelled form for positron emission tomography (PET) (105).

Pharmacological and clinical outlook

As shown in the previous sections of this review, potent and selective H_3-receptor antagonists are now available. Several indications for H_3-receptor antagonists have been proposed (Table II); three of them related to disorders of the CNS will be considered in more detail below. It is necessary to recall that functional H_3 receptors have been identified in the human brain (11, 15); moreover, their anatomical distribution in the human brain has been described (106).

Table II: Potential indications for H_3-receptor antagonists.

Dementia (Alzheimer's disease)
Epilepsy
Narcolepsy
Obesity
Schizophrenia

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Table IV Effects of H_3-receptor antagonists on learning and memory in rodents.

H_3-Receptor antagonists	Dose (mg/kg i.p.)	Species	Effect	Ref.
GT 2016	10 and 30	Mouse	Improvement in recall in the inhibitory avoidance and Y maze reversal paradigms Attenuation of the retention impairments induced by diazepam and scopolamine	120
	10	Rat	Improvement in performance in the 8-arm radial maze task	
GT 2016	7.5, 10, 20 and 30	Juvenile rat pup	Improvement in latency in a passive avoidance response paradigm	114
Thioperamide	2	Rat	Improvement in performance in a delayed non-matching to position task Improvement in acquisition and retention in a serial reversal learning task in the T maze	115
Thioperamide	15	Senescence-accelerated mouse (prone, strain 8) (SAM-P/8)	Improvement in latency in a passive avoidance response paradigm	116
Thioperamide[a]	20	Mouse	Attenuation of the scopolamine-induced learning deficit in the elevated plus-maze paradigm	117

[a]The effect of thioperamide reached a significant level when it was given in combination with the H_2-receptor antagonist zolantidine 20 mg/kg i.p.

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